

ADVANCES IN
AGRONOMY

DONALD L. SPARKS

VOLUME 136





VOLUME ONE HUNDRED AND THIRTY SIX

ADVANCES IN **AGRONOMY**

ADVANCES IN AGRONOMY

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VOLUME ONE HUNDRED AND THIRTY SIX

ADVANCES IN AGRONOMY

Edited by

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PREFACE

Volume 136 contains four outstanding reviews dealing with crop and soil sciences. Chapter 1 is a thoughtful review by one of the premier scientists in soil pedology, Richard W. Arnold, that provides perspectives on the National Cooperative Soil Survey. Chapter 2 is a comprehensive treatise on the CERES-Wheat, -Maize, and -Rice Models' Performances. Chapter 3 is a timely review on the impacts of herbicides on soil biology and function. Chapter 4 deals with how soil moisture and nitrogen application affect the performance of coffee seedlings.

I am grateful to the authors for their insightful and useful reviews.

Donald L. Sparks

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Perspectives About the National Cooperative Soil Survey

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Abstract

A discussion of nine tenets of pedology provides perspectives of the US soil survey program. The tenets are: paradigm of soils, scientific methodology, functional landscapes, soils as individuals, behavioral functions of soils, improving documentation, sharing pedological knowledge, reliability of information, and enhancing decision making. Highlights of each reveal progress, concerns, and opportunities. Accepting soil as a continuum rather than as individual entities may be the most important shift in our thought processes and understanding of the pedosphere. New technologies and changing social and political strategies suggest there is hope for a viable and sustainable Earth. Pedology can provide meaningful information and knowledge about soil resources.



1. INTRODUCTION

Soil Science has traditionally been an umbrella for soil physics, soil chemistry, soil microbiology, soil fertility, soil morphology, and soil technology. The area dealing with soils as entities in and of themselves has commonly been referred to as pedology (Arnold, 1983). Pedological activities in the United States have been prominent in the soil survey. The soil survey is the institutional construct that implements the concepts of the discipline of Pedology. After the land-grant colleges were authorized and charged with teaching agricultural knowledge, home economics, mechanic arts, and similar job training skills, the US Weather in 1894 created a Division of Agricultural Soils (Helms et al., 2002). A bit later agricultural experiment stations at those universities were federally funded and soon began the long-standing partnership of federal and state agencies and organizations. Since 1899 the partnership in soil surveys has been called the National Cooperative Soil Survey (NCSS). When the Soil Conservation Service was formed in 1935 their soil surveys were primarily for privately owned farms rather than the county soil surveys of the National Soil Survey group. All of the soil information was provided without charge and that is true today.

The mission of the NCSS has always been to help others better understand soils and use them wisely (Ableiter, 1938). This suggests that first one must know something about soil; what they are, how to recognize them, where they are, how and when they are formed, how they function, and their qualities and suitability. During attempts to learn and inform others about what had been discovered, there was awareness of the fragility of soil ecosystems and how human survival has been influenced by the improper functioning and use of the ecosystems (Lowdermilk, 1953). Consequently it became important to save these resources and use them wisely.

There are many perceptions and even definitions of what pedology is and has been (Brevik et al., 2015b). In the United States there is a century plus of events, personalities, results, and opinions of what happened and is happening. For me the driving force behind this history has been the positive attitudes of pedologists about what they call soils. To observe, study, model, and delineate similarities on maps is exhilarating. It is something real; it is not menial work; it is exciting and important, yet the details are mostly unknown. I like this quote of Werner Heisenberg, a theoretical physicist, “What we observe is not nature herself, but nature exposed to our method of questioning.”

Why perspectives about soil survey? For me they are evaluations of relative significance because we speculate about what we observe, describe, measure, and integrate into models. Consequently perspectives are viewpoints about what and why we do what we do. I was impressed with articles by Kellogg (1959) and Cline (1961) because I felt I was being talked to.

As a pedologist I believe that the truth is in the soil itself; it contains records of what happened. Most records are palimpsest where part of prior results are removed or erased and newer ones recorded over them. The real history of a soil is complex and not known with a high degree of certainty. Rather there are acceptable connections and relationships that enable us to think of soils as small individual volumes whose presence is a miniscule part of a continuum in space and time that is referred to as the pedosphere. Pedology is a subdiscipline of Soil Science; it is an interpretive venture into the existence of surficial earthy materials that we call as soils.

It is a probabilistic world; all measurements contain uncertainty. Measurements do not include value judgments. Numbers to not care and soils do not care, people do. Quality is a value judgment about being meaningful and is subject to all the vagaries of human thought about values. The basis for judgment is purpose. There can be multiple judgments of the same relationships depending on the purposes. All of this is certainly true for Pedology, the philosophical core of Soil Science.

When you stand in a field and look around, you see land surfaces, vegetation, sky, and maybe some human structures. We can't see below the ground surface and really know what is there. Road cuts, quarry faces, and pipeline trenches permit us to see 2D patterns, which we also try to visualize as 3D images. In other places we dig pits and can see and touch textures, colors, layers, and other features, which we extrapolate as parts of our mental models of the soils and their variability in a limited space. We understand some places better than others. Developing working models of soil-landscape relationships is critical to extrapolating point observations to the features of landscapes.

An interesting formulation of the thought processes in soil survey has been suggested by Bui (2004).

Soils provide and support many interpretive functions in ecosystems. All soil functions are environmental because soils are integral parts of terrestrial ecosystems. Important ones include biomass transformations, partitioning of water, regulation of fluxes, providing habitats, and other uses. Each specific function of soil can be stated as a purpose for an individual or group of users.

In 2001 the World Resource Institute noted that the challenge of civilization was to reconcile the demands of human development with the tolerances of nature. The Earth's capacity to produce food and other vital environmental functions under prevailing conditions is constrained by soil qualities, climatic conditions, and applied land management strategies. Yes, indeed, there are thresholds and limits!



2. TENETS OF PEDOLOGY

Let us examine the tenets of pedology. They are concepts which we believe and which guide our actions as pedologists. These tenets are perspectives about our science, important skills, field activities, interpretation of soil attributes, effective communication, and exploration of options ([Arnold, 2003](#)). Starting with the present we can describe kinds of changes that have taken place and serve as a basis for looking ahead.

1. The current paradigm of pedology is based on assumed connections among environmental factors (climate, organisms, parent materials, relief, and time) and biogeochemical processes, which can and do result in an altered surficial mantle recognized as the pedosphere. It embraces time as geologic age and temporal processes, both being irreversible.
2. As a subdiscipline of Soil Science, Pedology employs the scientific method in the study of uncontrolled experiments of the pedosphere. Evaluating working hypotheses is essential to field inventories.
3. Spatial relationships of physical and structural features of soils and their landscapes are needed to develop mental models of soil patterns that guide the preparation of maps.
4. For ease in managing concepts and data the continuous soil cover has been conceptually divided into units with limited variability that can be described, defined, classified, and used in naming delineated segments of the landscape. Generalized concepts at medium scales are often shown as 3D models.
5. It is crucial to continue improving the documentation and knowledge of space, time, and interacting relationships between: soil forming factors and soil forming processes; landscape history and present landscape segments; existing soil properties, temporal behavior, and degree of limitations; and models and reality.
6. Many functional relationships of soil attributes and current environmental conditions are consistent enough to describe soil behavior and

to predict responses to use and management. Attributes are interpretations of soil properties related to functions such as water holding capacity, fertility, erodibility, drainability, and structural stability. These interpretations rely on the knowledge and skills of many nonpedological specialists.

7. Our mission is to communicate effectively with users to help them better understand soils, their properties, functions, and behavior, which will assist them make informed land use and environmental decisions.
8. We are responsible to explain the reliability and limitations of our knowledge of the pedosphere including its formation and responses to changing environmental and social conditions, and our abilities to generate, describe, and provide soil resource inventories.
9. Future decision making can be enhanced by: training and use of new technologies in all aspects of pedology; explaining our rationale for decisions; packaging information for specific needs of the users; describing impacts of decisions made when using such information; and advocating stewardship of resources.



3. DISCUSSIONS

I am trying to bring together my impressions about the NCSS rather than details of its development over time. There are several very informative and useful documents that lead to meaningful literature. One of them is a collection of articles written by [Simonson \(1989\)](#) highlighting events from 1899–1970 that was published by the International Soil Reference Information Centre in Wageningen. A well-documented review of soil surveys and maps by [McCracken and Helms \(1994\)](#) was published by the Cornell University Press in Ithaca. Another chronology and collection of articles edited by [Helms et al. \(2002\)](#) and published by Iowa State University Press, Ames includes profiles of people and research related to the US soil survey. Finally two useful annotated bibliographies have been prepared by Brevik and coworkers. [Brevik et al. \(2015b\)](#) is about pedology and covers many aspects of soil surveys and [Brevik et al. \(2015a\)](#) is a bibliography and discussion on soil mapping, classification, modeling, and future directions published in *Geoderma*. I have not repeatedly indicated these references in the text. Throughout the Discussion and Visions sections I refer to specific articles that have influenced my viewpoints.

3.1 The Paradigm of Soils

When the cooperative soil survey began in the United States in 1899 most of the people had backgrounds in the geological concepts of soils and were interested in applying their knowledge to agriculture. That is, soils were considered to be straight line weathering products of the parent rock (or its derived earthy products such as glacial till or alluvium). This was the paradigm and it affected the attributes associated with soil properties. There was some animosity between Whitney, the Director of the Bureau of Soils, who emphasized the texture of soils as being the key to their productivity, and Hilgard, Prof. in California who emphasized the importance of climate and vegetation in the development of soil properties. A legacy of Whitney was the use of 1:63,360 scale USGS topographic maps as base maps for soil surveys.

This early period utilized an agrogeological approach to soils. When Marbut became the Chief Scientist he continued to use a similar approach. Coffey in 1912 mentioned the Russian concepts proposed by Dokuchaev but was politely ignored. Later Marbut read Prof. Glinka's book about soil types and their formation and published a translation in 1927. He began to emphasize that soils were independent of geology and could be studied for themselves. He mentioned 10 properties of soils that were relevant to understand soils. He used the concept of a "mature soil" as a meaningful reference for comparing soils. A mature soil was one whose properties expressed the impacts of climate and vegetation.

The first World Congress of Soil Science was held in Washington in 1927. As the lead speaker Marbut offered a definition of soil that he said had no element of theory in it and presupposed no process nor assumed any cause of the soil facts on which it was based. His definition was, "The soil consists of the outer layer of the earth's crust, usually unconsolidated, ranging in thickness from a mere film to a maximum of somewhat more than ten feet, which differed from the material beneath it, also usually unconsolidated, in color, structure, texture, physical constitution, chemical composition, biological characteristics, probably chemical processes, in reaction and in morphology." (Arnold, 1983)

The Russians presented 13 volumes of Russian Pedological Investigations at the Congress, 6 of which pertained more to soil survey. They were good summaries of the ideas of Dokuchaev, Sibirtsev, and others, which enabled many scientists to be aware of their progress in pedology (genetic soil science).

Kellogg was appointed as Chief Scientist in charge of the soil survey in 1934. Soon he wrote about the “normal soil” and functionally related it to soil-forming factors. He further stated that the principles of geography associated with the modern concept of the soil as a dynamic natural body in equilibrium with its environments lead us to consider both the destructive results of weathering and the constructional forces of biology. He believed that the whole process of soil genesis was one of evolution, together with the development of the entire landscape, of which it is a part, and that age in a relative sense was important. Thus time as an irreversible concept of processes and evolution was relevant to our understanding of soil landscapes.

Textbooks were beginning to reflect these ideas but it was not until after World War II that Jenny’s book on the formation of soils made a huge impact on American soil science (Jenny, 1941). He presented the functional relationships as $S = f(cl, o, r, p, t)$. Soil was considered to be a function of the interactions of climate, organisms, relief, parent material, and time. Even today this is the fundamental basis for talking about soils.

The concepts of Dokuchaev as espoused by many throughout the world gave rise to genetic soil science as a new and separate science based on the independence of soils as natural entities (Gerasimov and Glazovskaya, 1965). The science of soils as developed in the United States has had a strong association with agriculture and the production of plants of interest to society. For some this is agropedology, soil for the sake of agriculture, rather than pedology, which deals with soil for the sake of soil.

In a broader context, the pedosphere is the interface of the lithosphere, the biosphere, and the atmosphere. Occasionally the interface is also with the hydrosphere per se. The surficial materials altered by the processes of pedogenesis give rise to the pedosphere as distinct from the more comprehensive geoderma, which is the geologic cover of the Earth’s terrestrial area.

Our paradigm is a nice story and it allows us to imagine sequences of events of how and when landscapes form and evolve and how it might be possible to develop the altered properties of earthy materials we call soils (Buol et al., 2011). The fallacy is that we cannot readily grasp the enormity of such sequences or the utterly desolate conditions that had occurred time and again.

All of science needs and uses paradigms to guide us and help us accept that our knowledge is still incomplete. Brevik and Arnold (2015) suggest that the resurgence and expansion of interest in pedology and the many uses of soil information has not yet culminated in a paradigm shift.

3.2 Scientific Methodology

Research consists of those activities that involve the use and application of the scientific method to search for solutions to problems of scientific merit. Basic research is commonly thought of as investigations to determine fundamental properties of our universe, and applied research more often deals with the integration and use of existing information in solving pragmatic problems. In a physical sense, most activities of general interest to society do not attempt to find basic laws and theorems.

Research has several stages as the scientific method is being used. The stages include: collecting information or data, classifying and organizing the information, developing ideas (hypotheses) about relationships among the data, making predictions based on the hypotheses, and evaluating the results relative to the proposed predictions. Based on these results the original hypotheses commonly are modified and adjusted to provide more precise or accurate predictions for further testing and use.

A major distinction in developing and testing hypotheses occurs when controlled versus uncontrolled experiments are involved. A controlled experiment consists of systematic variations in treatments applied to a relatively homogeneous population. The objective is to evaluate results, which can be attributed to differences in the treatments. An uncontrolled experiment is the one in which the experimenter has little or no control over the treatments and only limited control (or knowledge) of the population. These experiments have already been run.

It is rather well known that if a landscape is adequately sampled, the results are differences that can be interpreted as differences of the intensity and the interactions of the soil forming factors (treatments) with the landscape. We recognize these differences as soils and as more observations are made, distinct trends and patterns within the uncontrolled experiments are detected.

Most laboratory measurements of soil samples follow agreed-on procedures of analysis; however, new methods are continually being introduced which enable us to refine and extend the results.

The information from uncontrolled geomorphic and pedologic experiments is organized into meaningful associations, which then become working hypotheses about soil formation and soil distribution. For histories more complex than we often understand or synthesize it is important to learn from geologists and their results (Holliday, 2006). Stepped landscapes hold many details of both landscape and soil evolution. Some even relate to sea level changes during the Pleistocene.

The scales of observations, the tools for examining soils, and the concepts of soils and their functional relationships have been modified over and over again throughout the development of the soil survey. Differences in intensity of land use such as grazing and cropping or irrigated and nonirrigated have commonly influenced the degree of exploration. Unfortunately our inability to link scales of maps of soil inventories has hindered our understanding of Nature. It is easier to generalize from specific details than vice versa.

Traditionally government-owned lands including parks, wilderness areas, reserves, forest and grazing lands, as well as tribal lands of Native Americans did not have the same support and attention as privately owned agricultural lands. This was related to the laws and regulations authorizing and supporting soil surveys.

3.3 Functional Landscape Relationships

Unraveling the results of uncontrolled experiments is the process in soil survey known as “legend building.” The relationships are working models that are hypotheses to be tested and refined. Soil survey is a dramatic example of applied research based on uncontrolled experiments with the results being the variability of soil themselves.

The basic premise of soil development appears simple and it is, as are many fundamental philosophical bases of scientific disciplines. The ramifications are astonishing. Each factor has a geographic or spatial distribution that permits hypotheses to be developed and tested for the accuracy of the predictions. There are many working models used in pedology, some are very good and others are in the initial stages of development and testing. A wonderful collection of hundreds of diagrams and explanations of such models are provided by [Schaeztl and Thompson \(2015\)](#) who also included more than 4500 references and a large glossary of terms.

Each relationship of sets of soil properties to specific landscape positions is, in fact, a hypothesis. The arrangement of the relationships observed in one location are thought to be representative of other areas and thus the working hypotheses are used as predictors of soil patterns at other sites. Legends for mapping the landscape segments are built in this manner and tested by the mapping of similar landscapes. Observations, random or systematic, are repeated again and again to validate interpretations of some of nature’s uncontrolled experiments.

If the experimental results do not refute the original proposal then the model is judged to be satisfactory for further use and testing. If the observations at a new site challenge the original prediction by differing

significantly, then a search must be made for an explanation of the features observed.

When a sufficient number of working models of a general area are developed and tested, the rapid assessment of the area can begin. Field mapping is the application or implementation of the working models. The number of models necessary to conduct field research depends on the objectives of the survey and the requirements for soil information.

As technology has introduced new techniques and procedures of measurement there have been important changes in how soil properties are described and interpreted (McBratney et al., 2000). In former years X-ray analysis enabled clay minerals to be studied in more detail, micromorphology examined the spatial relationships of small places, C14 and other isotopes refined dating of events and features in soils, and satellite data and images provided new opportunities for pattern recognition and improved basemaps. Today proximal testing of profiles in the field provides continuous distributions of many properties that open many possibilities for our understanding (Viscarra Rossel et al., 2011).

3.4 Soils as Individuals

Sets of facts are commonly used to characterize objects of interest. Relationships among the measured facts provide a basis for classification. The ideas or concepts that permit the human mind to perceive order and causal relations are, therefore, the basis for arbitrarily defining and naming parts of the real world and developing classifications that assist in consolidating such information into abstract models of the complex world about us.

Marbut pointed out that the work of creating the ultimate soil unit, as it existed in 1921, was done by the Americans (Arnold, 1983). The soil man, in his opinion, had to determine what features of soil have been acquired during their development as soils and what features had been inherited from the geological formation, which furnished the soil material. The soil man had to define the soil unit in terms of soil characteristics; he had to create the soil unit. Marbut concluded that the recognition of soil horizons and the description and identification of soils on the basis of the number, character, arrangement, and composition of horizons constituted probably the most significant contribution to soil science that had been made by soil survey.

Guidance to field parties was issued as part of the annual report of the activities of the soil survey. These served as the standards for conducting the surveys. Kellogg prepared a *Soil Survey Manual* in 1937 detailing the procedures and rationale for the description, mapping, and interpretations of soils.

The utilitarian land surveys of the Soil Conservation Service also had a *Field Manual*. When the two surveys were combined in 1951 a new revised *Soil Survey Manual* was published. This provided the world with a good description of how the surveys were being conducted in the United States.

There is an apparent dichotomy of thought when considering soil geography. On one hand, soil is thought of as a continuum of surficial material that meets the definition of soil; however, in practice the landscape is segmented into different soils. As with any method of segmenting a continuum, attention is focused on the limiting profiles or boundaries produced by applying class limits to the continuum. For most purposes, soil is thought of as a collection of natural bodies, which focuses attention on central or typifying concepts of the natural bodies. In this perspective, soils are described by a range of properties deviating from a central concept and, as such, are natural bodies not only as profiles but also as landscape segments occupying space.

This overlapping of geography implies that not only are soils spatially distributed but they also form a continuum of functional relationships in landscapes. Within this continuum no two spots have exactly the same combination of interactions of factors and processes, thus geographic variability is inherent in this model of soil.

An ideal basic unit for classification would be an object which is observable and measurable in three dimensions and include the whole vertical thickness of the soil; independent of all taxonomic systems; have clear boundaries even though arbitrarily fixed; and of a size convenient for study, measurement, and sampling. Most soil surveys are made with a particular taxonomy in mind that guides the naming of delineated areas and in some instances the location of boundaries that are not readily visible by external features (Cline, 1977).

A taxonomic soil class is a defined segment within a multidimensional array of sets of soil properties that are known from studying pedons or other sampling units of landscapes. As such, a taxonomic class is not a group of bodies of soil, but is a segment of a continuum of related soil properties with focus on the defined limits that separate the segments.

However, to a soil surveyor a basic taxonomic class is generally viewed as a group of physical entities and even though the idea of the group is a concept or a model, the constituent bodies of soil are real things. The natural bodies of soil are being studied to determine acceptable relationships on which to predict their distribution and then the areas are classified and named with taxa that have predetermined limits. The art and scales used in map making

and the recognition of intermingled soil bodies having contrasting qualities preclude delineating areas containing the same limits of variability as taxonomic classes.

At our current stage of comprehension it is very difficult to aggregate the knowledge about soils and their landscapes obtained in large scale mapping into a hierarchical system of classification of soils as geographic entities.

The exact combination of physical, chemical, and biological reactions that have actually transformed materials over time into soil horizons of a specific soil can never be known with certainty. Many useful generalizations have, nevertheless, guided the efforts to organize the available knowledge of soils. In an attempt to emphasize the combinational aspects of processes, [Simonson \(1989\)](#) discussed the general concepts of gains, losses, translocations, and transformations. Thus by inferring the initial state of materials accumulated in a profile and observing the present state of soil, the overall net changes of development and combinations and rates of processes could be estimated.

The concepts of soil development are entrenched in pedological thought and have influenced most of the soil classification systems. Sandstone soils, granite soils, glacial soils, etc. provided specific provinces for the soil series in the early years of the soil survey. The shift to soil as independent natural bodies was a change to climate and vegetation groupings of the soil series and over time the Great Soil Groups such as gray brown podzolic, brunizem, and reddish chestnut were recognized as having features thought to result from particular pathways of development. The soil series and soil types were very much the property of field personnel, whereas the higher category groupings were the speculative domain of others. Advances in classification concepts and their use are recorded in systems published since 1909. The *1938 Yearbook of Agriculture* was devoted entirely to the soils of the United States. In 1950 the soil survey staff began to design a new comprehensive system of classification, *Soil Taxonomy*, which was published in 1975, updated periodically with keys, and again published as revised editions in 1999 and 2006.

3.5 Behavioral Functions of Soils

Humankind's connection and interest in soils have usually been with the growth and harvest of plants of interest. Good wheat soils or rice soils were recognized and used. Quality of soil has generally been considered as an attribute by many people. It is a judgment about the degree of usefulness or satisfaction of some situation or service; consequently society behaves as a customer of the goods and services provided by soil resources. Each land use

objective has a set of limiting soil attributes that are critical. They differ in type and level of detail among uses. The utility of a soil inventory depends greatly on the extent to which it permits one to identify those soil attributes that limit soil performance.

Cline (1981) mentioned five kinds of information that would be necessary to predict soil performance in an onsite appraisal. They are: land use objective for which soil resources are to be evaluated, level of detail of information required to evaluate soil resources for that objective, soil properties that would be critical for the projected land use, degree of limitations which critical soil properties would impose on that use, and effects of the geographic distribution of limiting soil conditions on the anticipated use.

Some soil properties have existed a long time or have taken a long time to develop and are thought of as “inherent” or inherited properties. Changes of these properties are imperceptible on the scales of time we are familiar with. Some soil properties change rapidly, for example, moisture and temperature change daily and seasonally and such changes are thought of as dynamic ones.

Soil quality relates to the functioning of soils, how well they perform a function, and what we expect them to do. It may be good, bad, or somewhere in between (Norfleet et al., 2003). Soil functions include biomass transformations (productivity), water partitioning and reservoirs (Lin et al., 2005), geomembrane filters and buffers, biological habitats, direct uses, and cultural support.

Agriculture, grazing, and forestry are dependent on the establishment, growth, and maturity of plants for the benefit of society. Kellogg eloquently and simply described the “ideal arable soil” as the one in which the needs of plants for physical support, nutrients, and water were appropriately balanced and maintained; was resilient against degradation forces; and was economically viable.

Soils mantle most of the Earth and are the interface between the atmosphere and the lithosphere and as such, they partition water from high areas to lower ones; from impermeable to permeable areas; and retain moisture according to their physical, chemical, and biological compositions. The influence of moisture through and within soils has been of major interest to society throughout the centuries. Draining wetlands, irrigating deserts, and diking and bunding other lands are examples of how man has modified this natural function of soils.

The pedosphere serves as a sensitive geomembrane at the Earth’s surface, which affects the transfer of air, water, and energy into and out of this thin cover. Solar energy would possibly scald plant roots and microorganisms if it

were not for the moderating effects of soils. The mean residence time of pesticides, herbicides, and other contaminants in soils enable more effective remediation measures to be devised and implemented. Soils are also recognized for their potential to affect the flux of greenhouse gases, both positively and negatively. The formation of an Environmental Protection Agency attests to the importance that our society has placed on soils as the protective geomembrane of the Earth.

Soils are home for many macro- and microorganisms. Some complete life cycles take place within the soil at varying time and space scales. Soil fertility revolves around healthy, thriving communities of microorganisms. Adaptation to harsh and inhospitable environments is also common thus soil, by most definitions, must contain or be capable to support biological activity.

Throughout world history soil has been an important construction material used to build houses, roads, fortresses, and dams and support the infrastructure of society. It is so common that often soil is overlooked as vital for these functions. Soils also serve as the waste disposal receptacles for the refuse of evolving societies. Learning which soil properties are critical and limiting for projected land uses is a never-ending responsibility.

Indigenous people have traditionally maintained sanctity of the Earth in their daily lives as they interfaced with nature. Urbanized people often lose touch with their cultural roots in the soil, and are only occasionally reminded when visiting a cemetery or a recreational wilderness. For most people soil is also dirt, which is a nuisance that needs to be washed out and removed. Numerous archeological investigations reveal the evolution of man's efforts to live in harmony with his environment.

Modern society is more aware that soil quality is the capacity of a specific soil to function for a specific use and that there is both an inherent capacity based on the innate or inherited properties of soils and a dynamic capacity based on the changing conditions influenced by use and management.

The slowly evolving attitude in America about its soil resources is recorded in the soil survey reports that have been published since 1900. These documents follow the changes of models of soils and the importance of functions of soils, especially those of biomass transformations. In 1966 when Congress approved the "town and country" aspects of soil survey, a new era of assisting urban citizens began. No longer was the soil survey only for the benefit of agricultural pursuits.

Observing relationships of soils with patterns of behavior when used or treated in specific ways has been an ongoing activity during the course of the

survey. As correlations were established in one area for certain soils, they could be tested in other similar areas for consistency and accuracy. New machinery and techniques of managing land, new crop varieties, new fertilizers, herbicides and pesticides, and heightened awareness of the ravages of degradation all have been important influences on the relationships of soils with their behavior. In most instances site specific data is not available from soil surveys; however, it may be obtained from private specialists who have the time and expertise to make such investigations.

Good soils truly are a limited resource. Globally there is a desire and recognition that this resource should be carefully conserved and used in ways that are sustainably productive, environmentally safe, economically viable, and socially acceptable.

3.6 Improving Documentation

The NCSS holds regional meetings every 2 years alternating with a national conference. The topics for discussion are assigned by a steering committee of each conference and the participants contribute through written and oral correspondence with other members of their technical committee. The results of these deliberations are published internally for use by members and friends of NCSS. There have been many different technical committees over the years that have discussed soil properties, measures of reliability, procedures for updating surveys, marketing and research strategies, and items of a similar nature. Many of the reports have been archived on CDs and are available from the National Soil Survey Center in Lincoln, Nebraska. The National Soil Information System (NASIS) developed in the 1990s and maintained by NRCS benefits greatly from the input and testing by many cooperators.

Research is the foundation of a successful and viable soil survey program. In the United States, graduate students mainly at the land-grant universities working with their major professors have examined soils, their landscapes, and processes, and provided this information to the public.

In addition, the soil survey program of USDA initiated and sponsored numerous research projects including the major studies of soil and geomorphology in four representative areas during the 1950s, 1960s, and 1970s. They included coastal plain sediments in North Carolina, loess mantled glacial tills in Iowa, desert pediments in New Mexico, and various materials of the Willamette Valley of Oregon. Other studies were conducted in Puerto Rico and Hawaii. Concepts and approaches to field research are ongoing, both by the researchers of the soil survey staff and many students and

professors throughout the country. Since the centennial of NCSS in 1999, awards for outstanding pedologists have been recognized: Soil Scientist of the Year, Soil Scientist Achievement, and National NCSS Cooperator Achievement.

After the advent of Soil Taxonomy the continuing search for improvement of the system and the scientific relationships that support it were bolstered by USAID (Agency for International Development, Dept. of State) when they provided financial assistance to SCS for the Soil Survey Management Support program (SMSS, 1981). Workshops, training sessions, correlation trips, and other conferences enabled soil scientists from around the world to share and learn from each other. Important additions and modifications were proposed and accepted as Soil Taxonomy and its supporting laboratory data were enhanced during the 1980s and 1990s. Much of this information was used to expand the World Reference Base of IUSS to help correlate national soil classification systems. Other international cooperative ventures continue today.

During the economic boom of the 1980s agricultural production in the United States was enormous and at the same time there was increasing concern with environmental conditions both at home and abroad. Farm programs included new ways to reduce the overproduction, namely conservation reserves of land and protection of wetlands. Soil surveys were important in assessing the levels of anticipated production. As a result of the national and global interests in the environment, soil quality began to take its place with water and air quality. The NRCS established a number of centers and institutes to work on some aspects of these issues. One of them was the Soil Quality Institute which undertook collaborative research to provide a better description, definition, and monitoring of soil quality conditions throughout the country.

Advances in technology have been very useful in the soil survey program. Initially big changes occurred with cars and trucks instead of horses, air photos instead of topographic base maps, Munsell color charts instead of vials of different colored soils, more accurate photo mosaics and improved stereoscopic tools, updated map compilations and publications, and so on. It always seemed like a teenager “coming of age.”

Ground penetrating radar and electromagnetic bars let scientists look beneath the surface and estimate unseen properties more correctly. Data loggers facilitated transferring field descriptions to computer files and data tablets let surveyors in the field directly enter lines on base maps. New laboratory equipment has made it possible to shift from manual aspects to

machine-facilitated automatic procedures thus measuring more things more quickly. Proximal sampling of profile characteristics in the field can now provide data for more than 20 properties (Viscarra Rossel et al., 2011). As computer software and hardware advanced it was possible to join, digitize, and package soil maps on CDs and now let users make their own maps and texts using the vast databases.

The accuracy and precision of soil survey work hinges on the relationships that are discovered, reported, and used. There are many different kinds of relationships and some are very important for the survey. There are relationships concerned with soil genesis (Stockmann et al., 2011); between factors and processes and their interactions throughout the time; and those that are geographical whereby factors and landscape segments are correlated thereby improving soil mapping. Zhu et al. (2001) nicely described the limitations of conventional soil surveys and presented a new model, SoLIM, which facilitates the efficiency and reliability of updated surveys. When properties of soils and landscapes are linked to points and areas of landscapes, there are new possibilities to extrapolate and interpolate points to points and points to areas (Shi et al., 2009). The application of fuzzy sets and fuzzy logic to soil geography verifies some relationships and improves others.

Traditionally interpretations of soils were for production of common crops and simple engineering practices. Experiments by researchers and practical experiences of many others offer new and exciting avenues for extending and sharpening the insights about soil behavior and responses to management. The possibilities of checking and evaluating models with the realities of nature are always exciting. Brevik et al. (2015a) suggest that such opportunities are to be welcomed as they open the door for innovative and creative efforts to learn more about the pedosphere and its role in global ecosystems.

An important challenge for a soil survey program is finding an appropriate balance of the essential research and the conduct of operational aspects. Being aware of the different areas of relationships that backstop a viable soil program is a valuable component of any strategic plan. Available soil resource inventories, publications, and databases contain information, which makes them meaningful places to start.

3.7 Sharing Pedological Knowledge

Pedologists want to tell others what they have discovered about soils and the relationships of soils to the environment. There are many ways to communicate with each other—orally, written, with pictures, and by doing things

together. It has been the philosophy of pedology in the United States since its inception to provide information related to the growing of crops and which soils are better suited for certain crops. From the early reports on tobacco and grapes to the mixed farming, grazing, and specialty crops of today, there have been many kinds of interpretations prepared and presented to the agricultural community. We believe that people will make better and wiser decisions about land use if they have the best soil information available.

Soil survey reports have traditionally included general information about the properties and attributes of soils that favor ecosystems and conservation of the soil and water resources. Detailed scientific information about specific soil features, how soils form, and how they are distributed in landscapes are reported in the scientific literature, both here and abroad.

Because the soil survey program relies on standards to describe, identity, classify, and map soils, the preparation and availability of those standards have been common. Each *Soil Survey Manual* has provided the definitions and procedures used in carrying out the soil survey program. Operational details that are known to be successful are provided in the *Soil Survey Handbook*, the *Field Book for Describing Soils*, and in editions of *Soil Taxonomy* and *Keys to Soil Taxonomy* (NRCS, 2015). Today these and other documents are also available on the Internet. The soil survey staff of NRCS has been assigned the Federal leadership of the soil survey program for USDA and it maintains the records of the standards as agreed upon by the members of the NCSS.

A valuable component of the soil survey program is the outreach and extension to scientists in other countries. The United States has been fortunate to have had one of the largest, most successful programs in the world and it has continually offered its counsel and shared its standards with others around the world. The program in China was enriched by the works of Buck, Shaw, Pendleton, and Thorp during the 1920–30s and viable exchanges of scientists have occurred ever since (Gong et al., 2010). Working through the International Society of Soil Science many US pedologists have contributed to the expansion and dissemination of soil information. The *Seventh Approximation of Soil Taxonomy* was introduced at the World Congress of Soil Science in 1960 held in Madison, Wisconsin. Interactions with Iron Curtain scientists began in 1980 as work started on a world correlation scheme that eventually was introduced at the 1998 World Congress in Montpellier, France as the World Reference Base for classification. Today work on a Universal Soil Classification System is underway (Hempel et al., 2013) and in 2014 the International Union of Soil Science endorsed Soil Taxonomy as an approved system of soil classification.

Over the years the input and counsel from foreign soil scientists have been valuable contributions in the testing and modification of Soil Taxonomy. Many US soil scientists have had opportunities to visit other countries, examine and sample soils, and offer training in various aspects of the soil survey. Such activities are consistent with the mission of helping others understand soils and are important in the global strategy of the soil survey program.

3.8 Reliability of Information

The description of soils, their occurrence, and interpretations about their behavior have been cornerstones of the soil survey program. It was assumed and presumed that this information was the best available and that it could be used with confidence. Standards and accepted procedures were used in the conduct of the field and laboratory work of NCSS and when the results were published or became readily available, it was assumed that users were aware of the limitations of such information.

As statistics developed and matured in agricultural science, some concepts were applied to soil survey products. Laboratory data was quantified and easy to manipulate and analyze for statistical parameters such as means, standard deviations, and coefficients of variation. The precision of analytical data was used to evaluate new methods and equipment. Field observations such as thickness of surface horizons or depth to some underlying layers often had more variability than laboratory measurements; however, they could also be expressed in statistical terms if desired.

As soil series were described and redescribed, emphasis was given to a modal concept and the range of characteristics, which were thought to exist. Data from pedons were evaluated and the results commonly used in a descriptive paragraph provided a central concept for a particular soil series. This information was used extensively in the correlation process with eventual modifications to bring the properties in line with those permitted in Soil Taxonomy. This meant that the use of taxadjuncts became important components of soil map units. Taxadjuncts are very similar in the range of properties of a specific series but differ slightly (outside the range) such that they, strictly, are a different series but which are included because of their similarity in use, management, and location in the landscape.

Soil surveyors are familiar with the ambiguity between a taxonomic and a map unit. The former is a member of a class in a category of a hierarchical classification and often refers to a soil series at the lower category. A map unit is a geographic entity that has been named for the dominant soil component

or components but which includes small areas of other soils that cannot be readily delineated at the common scale of mapping. Most map units are multicomponent areas and this variability is explained again and again in discussions about the soil survey (Finnell, 2015).

Map unit delineations on maps vary in size and shape and there are no agreed-upon standards for their description or classification. Some interpretations of soil resources are improved as the individual components of map units are recognized, described, and interpreted. This has been recognized in precision farming and site-specific management practices. Quantitative measures of map unit variability are not very familiar to most users although probabilities of accuracy have been estimated and reported in some soil surveys. Fractal dimension values for map unit areas have not been demonstrated to be easily interpreted for collections of map units. The use of fuzzy sets and fuzzy logic allow one to examine possible membership of a data set into different classes and have been applied in developing some interpretations. Interpreting soils as continua presents challenges in description, naming, classification, and functional attributes.

It is unclear at this time what many customers and users of soil information want or are willing to use in their decision-making. Soil scientists would like to minimize the risk to consumers, at least the risk associated with the consistency of the scientific data and information provided. Economics commonly plays an important part in decisions about the use and management of soil resources, often overriding the scientific information. If there are acceptable probabilities of economic expectations, we are not aware of them as they might apply to soil surveys.

3.9 Enhancing Decision Making

The utility of a soil survey program is determined by the users of the information. Customers want to know which soils are suited for their purposes whether it is to grow plants or to hold up buildings and expect reliable information to make their decisions. Government programs designed to assist farmers, ranchers, and other users rely on data and their evaluation. Thus customers and stakeholders are critical to the success of any soil survey. Cline (1981, p. 7) noted that “people who are not expert in soil science are among the more important audiences for whom soil inventories are made. Yet these people have little or no basis for judging whether or not inventories important for their purposes are, indeed, suited to their objectives.”

Over the years flexibility in providing the needs of society has been a hallmark of the American soil survey. When people were uncertain of the products or the types of information needed, the scientists in the NCSS provided the results in published soil reports for survey areas, usually counties. In arid regions, data was interpreted mainly for grazing and irrigated and nonirrigated farming. In humid regions, data was interpreted for enhanced nutrient and water management to conserve the soil resources. Today that is history because now a user can become involved in generating maps and interpretations from large databases maintained by members of the NCSS. It has been the policy to be aware of the needs and desires of society and to provide the best information that is readily available or can be obtained in a reasonable manner.

The standards for making and interpreting soil surveys have always been published and made available to members of NCSS and now to the public on demand via the Internet. The *Soil Survey Manual*, *Soil Taxonomy*, NASIS data sets, and guidelines for many analyses and interpretations are packaged for broad use and available on the Internet at www.nrcs.usda/soil.survey.technicalpublications.

Experiences with the development and use of a soil classification system have demonstrated that people who are told the reasons or explanations for the selection and use of criteria are more comfortable. They can follow the line of reasoning and arrive at the same conclusions with the same information. Letting people make their own interpretations of soils required a systematic reorganization and treatment of the data used by soil scientists. NASIS was designed to let many people use the soil information and to provide their own criteria for evaluating the data and judging its appropriateness for their purposes. Similar approaches are now being used in many parts of the world, although there is not yet a globally accepted system for sharing and managing such data.

Most decision makers want to understand the impacts of their decisions. This is also true for those who want to use soil information. Earlier interpretations were often presented as three classes, red, yellow, and green similar to traffic lights suggesting stop (don't do it), be cautious, and go for it. The implications were usually as generalizations. Soil quality and multiple functions of soils are relevant in community, watershed, and state planning perspectives; consequently alternatives are as important as the impacts of a single use at a single site. Likely there will be many modifications and approaches as soils and environments are understood in more comprehensive ways.

The soil survey has learned some valuable lessons about the soil resources that they describe, map, and classify. Fewer really good soils exist at present than previously imagined. The soil resources of the United States are phenomenal; however, they are not unlimited. Worldwide there is a scarcity of high quality environments, which can provide food products for an expanding world population. There are soils whose productivities are fragile and easily degraded. Soil abuse through human history has shown how some soils are lost to benefit humankind and how costly it is to try to reclaim lands that have been misused, whether by accident or by design ([Lowdermilk, 1953](#)).

I am often reminded by Prof. Buol that a good soil has applied amendments that allow it to provide expected response and behavior whether the concern is fertility, moisture retention, or even foundations for buildings or other structures. Aha! memories of Kellogg and the “ideal arable soil.” “Good” is a subjective evaluation—a judgment, a viewpoint that changes as we, ourselves, evolve.

Before looking ahead let me remind that we do not own the soil resources, nor are we the keepers of them. Our current knowledge indicates that a sustainable future will not be a reality unless global inhabitants know, understand, care, and take actions to conserve the precious pedosphere on which they depend ([Molitor, 2003](#); [Mellody, 2010](#)). Promoting stewardship tenets and actions are vital and essential.



4. VISIONS BEYOND THE NEAR HORIZON

There are problems both on the near and far horizons. Concerns are both about pragmatic solutions for current problems and scientific solutions for underlying fundamentals that separate our minds from realities of nature.

The near horizons have visions about the survival of humankind and an establishment of a civilization that lives in harmony with nature rather than controlling it. There are mainly issues of population, food, shelter, health, education, realistic economics, and global involvement. Mankind’s abuse of soil resources has been historic and devastating as the “tragedy of the commons” reveals. Behaviors of soil resources dominate the interdisciplinary actions of soil science with other sciences to better understand the challenges of climate changes and also anticipate compromising actions of governmental bodies to develop a meaningful global habitat for all participants. These visions are here today; however, the solutions likely lie beyond the far

horizons. Sustainability is not a technological issue; it is a human rights and morality issue.

What you observe about the NCSS on the near horizon is a wonderful start of the hopes for the 21st century (Mausbach, 1998). During the International Year of Soils there were activities dealing with most aspects of pedology. They include new concepts and innovations, new technologies to test and apply, better databases, new minds to train, old minds to learn from, improved communications, and expanding interdisciplinary collaborations. And the best vision is that the truth of soil is in the soil itself.

In the realm of pedology there are continuing problems with how our minds work versus the realities of how nature operates. We prefer simplistic arrangements of information and knowledge. Often our pedological schemes are fractal and subfractal taxonomies used with map representations of what we extrapolate as models of our discipline. Does this imply that we deal with randomness or is it an artifact of how we react when trying to organize and transmit our findings?

We realize that we cannot reliably link our maps at different scales, which suggests we do not yet know the properties or attributes that are such links. It appears that a resolution of the ambiguity and dichotomy of believing the soil cover is a continuum and not a collection of central concepts (taxonomic classes) will facilitate communication and sharing of data and information (Libohova et al., 2010). As of now no solution has been globally accepted by our scientific community.

The evolution of geologic and geomorphic components of landscapes, regardless of scale, is generally relevant to unraveling the history of soils in an area. Will that ever change; will we find new ways to comprehend soils and their spatial distributions?

Each of us has been here before and we know what is coming. It is almost a litany.

Visions on the far horizons are those of hope. Hope that new technologies, continued evolution of *Homo sapiens*, and new mindsets will consolidate a history of learning and doing pedology that will let it continue as the philosophical basis for soil science. The soil survey of the future waits on the far horizon. For you, the leaders of tomorrow, keep in mind what Peter Drucker, a management consultant and author, once said, "Rank does not confer privilege or give power. It imposes responsibility."

And as a final perspective, I would like to share the following poem (Arnold, 2006, p. 21):

Go Toward the Light

*It is dark now, not only does the moon not shine
The cleverness of world leaders still covet 'what's thine'
Exponential growth has captivated a worldwide soul
Unbridled consumption fosters poverty and takes its toll,
Technology and runaway economics say 'that's mine'*

*We exist on a planet with the utmost of extremes
Starvation and malnutrition haunt like midnight screams
Excesses of materialism abound for over-developed ones
Unrealistic cities and roads, luxuries covering naked bones
Dehumanized entities have lost touch with nature's dreams*

*It is dark now in the hearts of a myriad of bewildered beings
'Nearsightedness' is when visions see only such things
Immediacy will never offer us intergenerational equity
Nor safe promotion and protection of environmental quality
Moving fast over global limits toward the disaster it brings*

*We exist on a planet where the candle of hope is aglow
Although the flames of faith, truth and love no longer show
Hope remains bright in the hearts and souls of humankind
You and I and those around us are parts of a collective mind
With hope we can relight the other three that they may grow.*

*With the power of vision to see far beyond the obvious
The horizon of this new found wisdom is truly glorious
A world once again within its limits to sustain us all
Sufficiency for humans and for all things, 'big and small'
Let Faith in the Truth of Love make the dark obnoxious.*

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A Comprehensive Review of the CERES-Wheat, -Maize and -Rice Models' Performances

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Abstract

The Crop Environment Resource Synthesis (CERES) models have been developed and utilized for the last 30 years to simulate crop growth in response to climate, soil, genotypes and management across locations throughout the world. We reviewed 215 papers found in the literature that contained field observed data where the CERES models were tested. Over 30 simulated variables of the CERES models have been

tested in 43 different countries under various experimental treatments. Across all testing conditions, the CERES models simulated grain yield with a root mean square error (RMSE) of less than 1400 kg/ha (~10% relative error, RE), 1200 kg/ha (~20% RE) and 800 kg/ha (~10% RE) for maize, wheat, and rice, respectively. Phenological development was simulated with less than 7 days difference from the observations in most studies. The CERES models simulated aboveground biomass, harvest index, evapotranspiration, and soil water reasonably well too. The simulations of grain number (up to 4340 root mean square error, RMSE), grain weight (up to 22% error), intercepted photosynthetically active radiation (IPAR, up to 0.41 MJ/plant), leaf area index (LAI, 31.9% error), soil temperature (over 10°C difference), and nitrogen (N) dynamics (up to 80% error) were less accurate. In fact the average error of CERES model simulations tends to be higher under marginal crop growing conditions such as extreme heat or cold, water and nutrient deficit conditions.



1. INTRODUCTION

Food security is one of the most important ecosystem services offered by agriculture (Reid et al., 2005; Zhang et al., 2007). Due to the high demand for food, there has been an expansion and intensification of agriculture (Matson et al., 1997). Considering the increasing food demand by the rising population, the agriculture sector is facing the big challenge to increase food crop productivity. Since about two-thirds of the total daily calorie-intake is from the three staple grains of wheat, rice, and maize, increasing their yields is mandatory (Cassman, 1999). Although we have witnessed extensive efforts in agricultural experiments that aimed at increasing yield and minimizing environmental impact, the results are often site specific and subject to spatial and temporal variability affected by weather, soil, and crop cultivars (Basso et al., 2011). This variability in space and time makes it difficult to transfer crop management information from one location to another for agricultural decision making (Jones et al., 1998). To understand the complex crop-soil-weather system and to facilitate farm-level decision making processes, crop models were developed to help provide the larger combinations of crop yield outcomes as influenced by variety and management for the high degree of variability in weather and soils than would be possible using trial and error experiments. The Crop Environment Resource Synthesis (CERES) models were developed in the early days of the information age. CERES-Wheat (Otter and Ritchie, 1985, Ritchie, 1985),

CERES-Maize (Ritchie, 1986, Ritchie et al., 1986b, Jones et al., 1986), and CERES-Rice (Ritchie et al., 1986a) were initially developed mainly to simulate grain yield, but later served also as a decision support tool when DSSAT became available (Jones et al., 2003). The CERES models are a process-based system that simulates crop growth and development on a daily time step. The major components of phenology, growth, soil water, and nitrogen balance enable the models to simulate crop yield, using the soil water and nitrogen dynamics to provide a limitation on yield. Maize, wheat, and rice are the most tested and used, but models of barley, grain sorghum and pearl millet are included in the CERES models (Ritchie et al., 1998).

While Timsin and Humphreys (2006) reviewed the performance of the CERES-Rice and CERES-Wheat models in the rice-wheat systems, there lacked a comprehensive reviews on the CERES model performance for staple crops. Therefore, the objective of this paper is to summarize published results of worldwide tests of the CERES-Maize, CERES-wheat, and CERES-Rice providing a review of (1) the crop and soil variables that have been tested for the models, (2) the conditions under which those parameters were tested, and (3) the accuracy of the simulated variables.

Testing of models involves comparison of simulated with measured results. When the model results differ from measurements, the apparent error can be a function of the inaccuracy of one or more of the model functions and the resulting feedback, but much of the difference can often be the result of inaccurate input data or initial conditions. The CERES models are intended to be applicable in any weather, soil, and management conditions for any cultivar in which the genetic coefficient information is known and should not require calibration in space and time. Some critical input information for CERES models is often not known and has to be approximated by various procedures. The greatest uncertainty of inputs has proven to be the depth of effective rooting, one or more of the genetic coefficients, and the initial conditions of the soil, water, and nitrogen. When crops are grown under unlimited water and nitrogen, the soil properties and initial conditions are less critical for more accurate simulation. If there is no independent knowledge about the genetic coefficients, the model is often calibrated to make the phenology and yield components match the data sets. The initial conditions are frequently identified by trial and error simulations or through sophisticated parameter estimation techniques to obtain better agreement with the final measurements.



2. METHODS

The reviewed articles were obtained from the ISI Web of Science database. All peer-reviewed articles in the database that met the following criteria were selected for this metadata synthesis paper: (1) written in English, (2) published by Dec. 2014, and (3) primary studies had both field observations and simulation results obtained from the CERES models.



3. RESULTS

A total of 215 field studies tested the three CERES models. Of these, 111 studies tested the CERES-Maize model, 104 studies tested the CERES-Wheat model, and 26 studies tested the CERES-Rice model. The CERES models have been validated in 43 countries across all continents, except Antarctic (Fig. 1). The model simulations have been tested under a wide range of climate conditions: monsoonal (Liu et al., 2013), semiarid tropical (Carberry et al., 1989), subhumid, subtropical (Behera and Panda, 2009), Mediterranean (Hasegawa et al., 2000), oceanic and

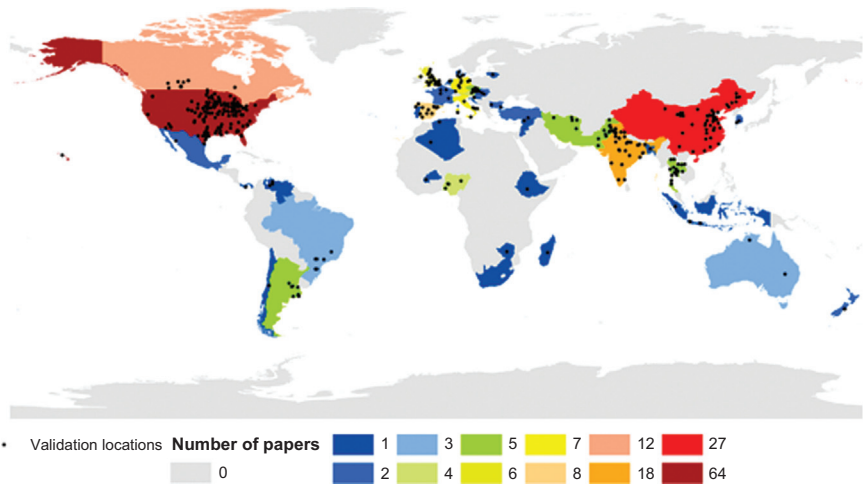


Figure 1 Locations where the CERES models have been validated and the number of studies in each model validation country.

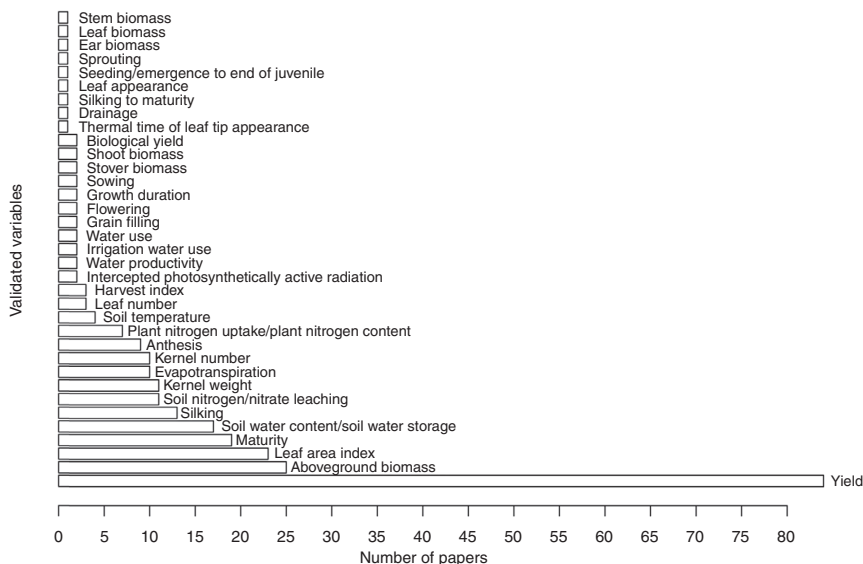


Figure 2 The number of research that validated variables in the CERES-Maize.

continental (Johnen et al., 2012), cold winter, dry summer (Thaler et al., 2012), and humid temperate (Otegui et al., 1996). The validation studies have been conducted extensively in the United States (64 studies), China (27 studies), and India (18 studies) under varied treatments, such as nonlimiting irrigation and fertilization; various irrigation water and fertilizer amounts, timings, and application methods; sowing dates; population densities; CO₂ concentrations; tillage methods; and management intensities (Fig. 1). Grain yield, aboveground biomass, leaf area index (LAI), anthesis, and maturity have been extensively tested. Variables regarding water balance (evapotranspiration and soil water content), nitrogen balance (soil nitrogen and crop nitrogen uptake and content), phenological stage (grain filling, silking, and panicle initiation), and other biomass components (leaf biomass, straw biomass, and shoot biomass; and harvest index) have been less extensively validated (Figs. 2–4).

3.1 Crop Phenology

3.1.1 CERES-Maize

The CERES-Maize model has been tested regarding sowing date (two studies), sprouting (one study), days from seedling emergence to the end of the juvenile stage (one study), leaf appearance (one study), flowering date

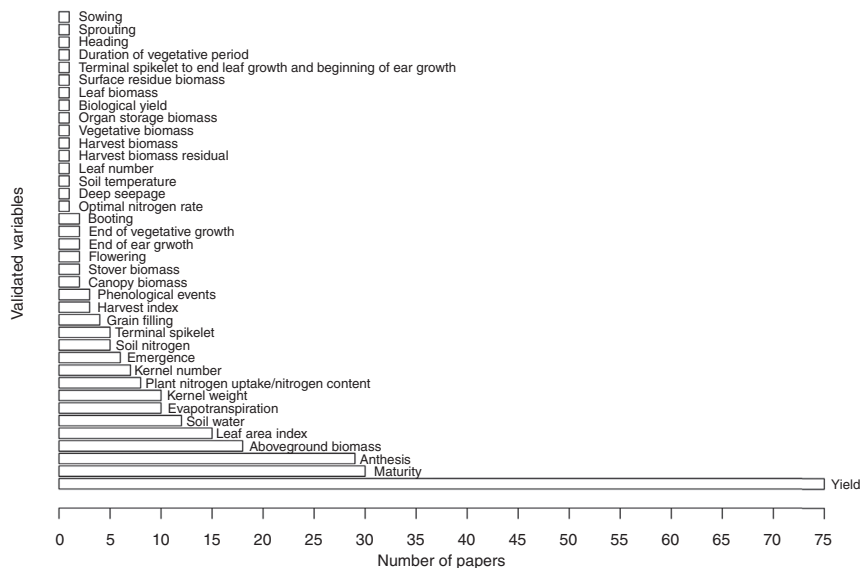


Figure 3 The number of research that validated variables in the CERES-Wheat.

(two studies), anthesis (nine studies), silking (13 studies), grain filling (two studies), silking to maturity (one study), maturity (19 studies), and growth duration (three studies) (Table 1 and Table 2). Strzepek et al. (1999) and Wang et al. (2012) showed that the simulated planting dates for maize grown in Iowa and Missouri (USA) and in one experimental maize station in China matched well with the recorded sowing dates, with at most 1 day of error. The predicted days from seedling emergence to the end of the juvenile stage in a 4-year simulation in Brazil were, on average, 3 days off from the observations (Liu, 1989). Hodges and Evans (1992) tested the leaf tip appearance variable and reported that the simulated days were delayed up to 15 days. Regarding the flowering date simulation, the RMSE was less than 4 days under the sowing date treatment in Portugal (Braga et al., 2008) and within 7 days of error in two US states (Strzepek et al., 1999). Anthesis date has been well predicted under full irrigation and moderate and severe water stress treatments in Italy, with simulation errors being within 6 days and percentage errors ranging from 0 to 2.8% (Ben Nouna et al., 2000; Mastrorilli et al., 2003). In Brazil, anthesis date simulations across irrigated and rain-fed conditions had a normalized RMSE of 1.6% (Soler et al., 2007). Under irrigated and rain-fed conditions in a county in Georgia (USA), with fertilization (141 ~ 219 kg N/ha application) and planting date (three dates across Mar.)

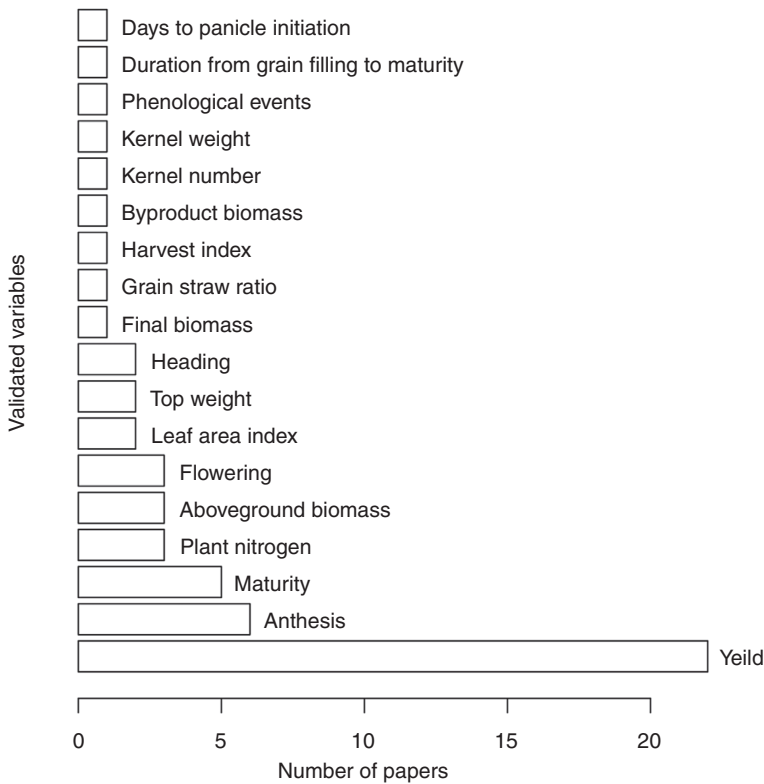


Figure 4 The number of research that validated variables in the CERES-Rice.

treatments, the anthesis date simulations were within 9 days of the measured data (Persson et al., 2009). The simulated days to anthesis for fertilizer trials in Ethiopia were within 7 days (Kassie et al., 2014). The CERES-Maize model has not only estimated anthesis date reasonably well for agriculture experimental stations for over 11 years in Georgia (RMSE = 3.5 days) and Louisiana (RMSE = 4.3 days) but also captured the inter-annual variability (Tsvetsinskaya et al., 2003). Under both irrigation and nitrogen application treatments in Iran and seven irrigation treatments in Pakistan, the days to anthesis simulations had a normalized RMSE of 2.3 ~ 2.6% and RMSEs of less than 2.2 days, respectively (Moradi et al., 2013, 2014; Mubeen et al., 2013). In terms of maize silking date simulation, the reported errors were within 4 days for simulations in Nigeria (Jagtap and Abamu, 2003; Jagtap et al., 1993), Brazil (Liu, 1989), and Venezuela (Maytin et al., 1995). The reported RMSEs were between 2 and 4 days for unfertilized maize

Table 1 Summary of the CERES-Maize model performances for phenology variable (excluding anthesis and maturity) simulations.

Treatment category	Variable name	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized	Silking	Croatia Nigeria Venezuela	Percentage error: 1% Error: <3 days	Vucetic (2011) Jagtap et al. (1993); Jagtap and Abamu (2003); Maytin et al. (1995)
Irrigated with a gradient of water/different scheduling time and well fertilized	Silking	United States, Italy	Differences: 0 ~ 4 days	Anothai et al., (2013); Ben Nouna et al. (2000)
Well irrigated and fertilized with a gradient of fertilizer(s)	Silking	Australia Nigeria	RMSE: 10.6 days Differences: 0 ~ 14 days	Carberry et al. (1989) Gungula et al. (2003)
Well irrigated and well fertilized only ^a	Grain filling date	Nigeria	Differences: 0 ~ 12 days	Gungula et al. (2003)
	Planting date	China	Difference: <1 day	Wang et al. (2012); Strzepek et al. (1999)
	Flowering	United States	Error: <7 days	Strzepek et al. (1999)
	Silking	United States Argentina	RMSE: 4 days RMSE: 6.5 days	Retta et al. (1991) Caviglia et al. (2013)
		China	Delay: 1 day	Wang et al. (2012)
	Grain filling	China	Error: 1 day	Wang et al. (2012)
	Growth duration	China	R: 0.99	Xiong et al. (2007)
	Silking to maturity	Brazil	Mean error: 0.5 days;	Liu (1989)
	Emergence to end of juvenile		Mean error: 3 days	
	Silking		Error: <4 days	
	Tip appearance	USA	Error: 15 days	Hodges and Evans (1992)

Irrigated with a gradient of water, not fertilized and other treatments	Flowering date	Portugal, Argentina, United States	RMSE: 6 ~ 8.26 days	Braga et al. (2008); Otegui et al. (1996); Tsvetsinskaya et al. (2003)
Sowing dates Planting dates, spacing Planting dates, fertilization	Silking	Argentina	RMSE: 4.3 days	Otegui et al. (1996)
	Flowering	Portugal	RMSE: <4 days	Braga et al. (2008)
	Silking	USA	RMSE: 2 ~ 3.4 days	Yang et al. (2009)
	Growth duration	Zimbabwe	Error: <3 days	Makadho (1996)

^aLiterature that did not include treatments were considered as “well irrigated and well fertilized”; for instance, data obtained from local reports.

Table 2 Summary of the CERES-Maize model performances for anthesis and maturity variable simulations.

Treatment category	Variables	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized	Anthesis	Brazil	Normalized RMSE: 1.6%	Soler et al. (2007)
	Maturity	Nigeria, Croatia	Difference: 1 ~ 2 days	Jagtap et al. (1993) ; Vucetic (2011)
		Nigeria	Percentage error: 2% Differences: 7 ~ 10 days	Jagtap and Abamu (2003)
Irrigated with a gradient of water/different scheduling time and well fertilized	Anthesis	Italy	Percentage errors: 0 ~ 2.8%	Ben Nouna et al. (2003)
		Pakistan	RMSE: <2.2 days	Mubeen et al. (2013)
		Australia	RMSE: 10.6 days	Carberry et al., (1989)
	Maturity	United States, Nigeria	Differences: <5 days	Anothai et al. (2013) ; Gungula et al. (2003)
		Pakistan	RMSE: 3.7 days	Mubeen et al. (2013)
		Italy	Error: 0 day	Mastrorilli et al. (2003) ; Ben Nouna et al. (2000)
Well irrigated and fertilized with a gradient of fertilizer(s)	Maturity	Australia United States, China, Ethiopia	RMSE: 10.2 days Difference: <1 day	Carberry et al. (1989) Strzepek et al. (1999) ; Wang et al. (2012) ; Kassie et al. (2014)
	Anthesis	Ethiopia	Difference: <7 days	Kassie et al. (2014)

Well irrigated and well fertilized only ^a	Anthesis	Nigeria, Brazil, Venezuela, Argentina, United States, China, Iran	Difference: <4 days Normalized RMSE: 2.35%	Jagtap et al. (1993); Jagtap and Abamu (2003); Liu (1989); Maytin et al. (1995); Yang et al. (2009); Wang et al. (2012); Moradi et al. (2014)
	Maturity	China Argentina	Difference: 1 ~ 3 days RMSE: 14.3 days	Wang et al. (2012) Caviglia et al. (2013)
Irrigated with a gradient of water and fertilized with a gradient of fertilizer(s) Other treatments ^b	Anthesis	Iran United States	Normalized RMSE: 2.61% Differences: 1 ~ 9 days	Moradi et al. (2013) Persson et al. (2009)
	Maturity	Portugal United States	RMSE: <4 days Error: 1 ~ 9 days	Braga et al. (2008); Persson et al. (2009)
	Anthesis	United States	RMSE: 3 ~ 4 days Tsvetsinskaya et al. (2003)	
	Maturity	United States, Argentina	RMSE: <6 days	Yang et al. (2009); Otegui et al. (1996); Tsvetsinskaya et al. (2003)
			Difference: 9 ~ 11 days ^c	Strzepek et al. (1999)

^aLiteratures that did not include treatments were considered as “well irrigated and well fertilized”; for instance, data obtained from local reports.

^bOther treatments included sowing dates, planting density, spacing.

^cAuthors pointed out that the large error might be due to the differences between simulated physiological maturity and observed grain maturity.

experiments in Argentina (Otegui et al., 1996) and sowing dates combined with spacing treatments in the USA (Yang et al., 2009). Seventy-five percent silking date simulations had RMSEs of 4 days in the United States (Retta et al., 1991) and an error of 1 day in China (Wang et al., 2012). While the good prediction of silking held for rain-fed conditions in Croatia (error: 1%) (Vucetic, 2011) and irrigation treatments in the United States (error: 0 ~ 4 days) (Anothai et al., 2013), the prediction was moderately accurate for nitrogen unavailability treatments in Nigeria (error of 0–14 days) (Gungula et al., 2003), well-irrigated and fertilized condition in Argentina (RMSE: 6.5 days) (Caviglia et al., 2013), and water stress treatments in Australia (Carberry et al., 1989). Regarding grain-filling validation, it was reported that the differences between the simulated and observed values were mostly 0 or 1 and sometimes up to 2 days under a high nitrogen application rate (90 or 120 kg/ha), but the difference was at least 4 days and sometimes up to 12 days under a low nitrogen application rate (60 kg/ha) in Nigeria (Gungula et al., 2003). Under irrigated treatments in China, the simulated grain filling date was only 1 day delayed as compared to the observed date (Wang et al., 2012). As to maize maturity, the simulated maturity date was reported to be very close to the observations across various sowing dates from May to Jun. in Venezuela (Maytin et al., 1995), across 40–100% full irrigation treatments in Colorado (USA) (Anothai et al., 2013), and under high fertilizer application rates (90 and 120 kg N/ha) in Nigeria (Gungula et al., 2003). Under water availability treatments in Italy, most simulated maturity dates were exactly the same as the observations (Ben Nouna et al., 2000; Mastrorilli et al., 2003). For fertilizer trials in Ethiopia, the difference between the simulated and the observed days to maturity was within 1 day (Kassie et al., 2014). Jagtap et al. (1993) and Vucetic (2011) also reported small errors in maturity date simulations under rain-fed treatments in Nigeria and Croatia, with 1 or 2 day differences and a 2% difference, respectively. The RMSEs for maturity simulations were within 6 days under the sowing dates between late-Apr. and mid-Jun. in treatments in Portugal (Braga et al., 2008), across irrigation treatments (irrigated at various rates and in various amount) in Pakistan (Mubeen et al., 2013), across four sowing date treatments in Argentina (Otegui et al., 1996), in agricultural stations in Georgia and Louisiana for over 11 years (Tsvetsinskaya et al., 2003), and at 11 locations in North Carolina (Yang et al., 2009). However, Carberry et al. (1989) and Caviglia et al. (2013) reported that the average RMSEs for maturity simulations across full irrigation and severe water stress treatments in Australia

(Carberry et al., 1989) and stress free conditions in Argentina (Caviglia et al., 2013) were over 10 days. Strzepek et al. (1999) and Jagtap (2003) reported that the simulation errors ranged from 7 days to 11 days in the United States and Nigeria, respectively (Jagtap and Abamu, 2003). Persson et al. (2009) simulated maize maturity dates across irrigated and rain-fed land with three levels of fertilization treatment and three planting date treatments in Georgia (USA), and the reported errors ranged from 1 to 9 days for three maize cultivars. Additionally, one study reported the days from silking to maturity in a 4-year study of a Brazilian maize cultivar and had a mean error of 0.5 days (Liu, 1989). The simulated growth durations matched the observations with high correlation coefficients of 0.99 for three production stations in China (Xiong et al., 2007) and less than 3 days of errors in Zimbabwe (Makadho, 1996).

3.1.2 CERES-Wheat

The CERES-Wheat model has been tested for sowing date (one study), sprouting (one study), emergence (six studies), booting (two studies), heading (one study), terminal spikelet (five studies), end of vegetative growth (two studies), end of year growth (two studies), duration of vegetative period (one study), flowering date (two studies), anthesis date (29 studies), grain filling date (four studies), mature date (30 studies), and other phenological events (five studies) (Tables 3–5). Sowing date and sprouting date were tested in Northwest China, with simulation errors of 0 and 2 days, respectively (Wang et al., 2012). Emergence date was tested under a well-irrigated and fertilized treatment with five sowing dates [day of year (DOY) 125, 128, 129, 164, and 296] in New Zealand. Delays in the simulated emergence date of two wheat cultivars were observed, except for the DOY125 treatment simulation. The average RMSE for the emergence date of the two wheat cultivars was 12.1 days (Porter et al., 1993). With 110.5 and 241 kg N/ha application treatments in Arizona (USA), the 50% crop emergence date simulation was delayed by 1 day (Thorp et al., 2010b). Chipanshi et al. (1997) reported that the ratios between the simulated and observed emergence dates for 30 years in a long-term agricultural site in Canada were from 0.47 to 0.77 (Chipanshi et al., 1997). When the CERES-Wheat model was used to simulate spring wheat growth in 24 sites across North America, including the United States and Canada between 1930 and 1954, the days from sowing to 50% seedling emergence were underestimated for 94% of the sites, with a RMSE of 5.8 days. The model concordance correlation coefficient and bias correction factor were 0.232 and 0.396, respectively

Table 3 Summary of the CERES-Wheat model performances for phenology variable (excluding anthesis and maturity date) simulations.

Treatment category	Variables	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized only	Emergence	Canada	23 ~ 53% earlier	Chipanshi et al. (1997)
	Terminal spikelet	Canada	36% earlier ~ 62% late	Chipanshi et al. (1997)
	End of vegetative growth	Canada	23% earlier ~ 44% late	Chipanshi et al. (1997)
	End of year growth	Canada	23% earlier ~ 43% late	Chipanshi et al. (1997)
	Grain filling	Canada	23% earlier ~ 41% late	Chipanshi et al. (1997)
Irrigated with a gradient of water/different scheduling time and well fertilized	Emergence	China	Difference: 0 days	He et al. (2013)
	Phenological events	India	RMSE: 4 days	Sarkar and Kar (2008)
Well irrigated and fertilized with a gradient of fertilizers	Emergence	United States	delay: 1 day	Thorp et al. (2010b)
	Phenological events	Canada	RMSE: 3.15 days	He et al. (2014)
Well irrigated and well fertilized ^a	Sowing date	China	Error: 0 day	Wang et al. (2012)
	Sprouting date		Error: 2 days	
	Emergence	United States	RMSE: 5.8 days	Wang et al. (2009)
		China	7 days earlier	Liu and Yuan (2010)
	Terminal spikelet	China	6 days earlier	Liu and Yuan (2010)
	End of vegetative growth	China	Difference: 1 day	Liu and Yuan (2010)
	End of year growth	China	Difference: 8 days	Liu and Yuan (2010)
	Flowering	China	R^2 : 0.66	Zhao et al. (2011)
		China	Difference: <7 days	Wang et al. (2012)
	Grain filling	China	4 days earlier	Liu and Yuan (2010)
	Phenological events	China	RMSE: 5.6 days	
Sowing dates	Emergence	New Zealand	RMSE: 11 ~ 13.2 days	Porter et al. (1993)
	Booting	United States	RMSE: 5.3 days	Xue et al. (2004)
	Heading	United States	RMSE: 4.8 days	Xue et al. (2004)
	Terminal spikelet	New Zealand	15 days earlier ~ 19 days late	Porter et al. (1993)
		United States	RMSE: 4 ~ 7 days	Xue et al. (2004)

Sowing dates, fertilization treatments and wheat rotation systems	Duration of vegetative growth	Czech Republic	Error: 3 ~ 4 days	St'astna et al.2002
Sowing dates with varied temperatures	Duration of grain filling	United States	RMSE: <5 days	White et al. (2011)
CO ₂ concentration	Booting	United States	2 days delay	Tubiello et al. (1999a)
	Terminal spikelet	United States	3 days earlier	Tubiello et al. (1999a)
	Grain filling	United States	1 day earlier	Tubiello et al. (1999a)
	Phenological events	Germany	Normalized RMSE: <15%	Biernath et al. (2011)
848 complied field dataset	Terminal spikelet to end of leaf growth	Germany	Error: <9.1 days	Johnen et al. (2012)

^aLiteratures that did not include treatments were considered as “well irrigated and well fertilized;” for instance, data obtained from local reports.

Table 4 Summary of the CERES-Wheat model performances for anthesis simulations.

Treatment category	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized	Australia	Difference: <7 days	Alexandrov et al. (2002)
	United States, Australia	RMSE: 1 ~ 6 days	Xue et al. (2004) ; Thaler et al. (2012)
Irrigated at varied rates (and timings) and well fertilized	United States	RMSE: ≥ 9 days	Xue et al. (2004)
	China	Difference: 0 ~ 2 days	He et al. (2013)
	Argentina	RMSE: 2.7 days	Savin et al. (1994)
Well irrigated and fertilized at varied rates	United States	Difference: <10 days	Thorp et al. (2010b)
	India	RMSE: 5.3 days, normalized RMSE: 7%	Timsina et al. (2008)
Well irrigated and well fertilized ^a	United States, United Kingdom, Argentina, Czech Republic, China	RMSE: 4 ~ 8 days	Ottman et al. (2013) ; Bannayan et al. (2003) ; Caviglia et al. (2013) ; Trnka et al. (2004) ; Liu and Tao (2013)
	New Zealand	RMSE: 8 ~ 22 days	Porter et al. (1993)
	Italy	Normalized RMSE: 6 ~ 8%	Dettori et al. (2011)
	China	Error: ≤ 9 days	Xiao et al. (2013) ; Liu and Yuan (2010)
	China	Relative absolute error: <12%	Tian et al. (2012)

Irrigated with a gradient of water and fertilized with a gradient of fertilizer(s) Not irrigated and fertilized with a gradient of fertilizer(s)	Mexico	Difference: 0 ~ 8 days	Lobell and Ortiz-Monasterio (2006)
	Spain, Bangladesh, India	RMSE: 4 ~ 7 days	Timsina et al. (1998) ; Abeledo et al. (2008) Saseendran et al. (2004)
	United States	6 days earlier ~ 2 days late than the observations	
	Germany	Percentage error: <7%	Bacsi and Zemankovics (1995)
CO ₂ concentration	United States	Difference: 1 day Normalized RMSE: 4%	Tubiello et al. (1999a) Tubiello et al. (1999b) ^b
Sowing Date	Algeria, United States India	RMSE: 4 ~ 5 days Difference: ≤9 days	Rezzoug et al. (2008) ; White et al. (2011) ^c Hundal and PrabhjyotKaur (1997)
Spacing	United States	Average error: 0 days	Tsvetsinskaya et al. (2003)

^aLiteratures that did not include treatments were considered as “well irrigated and well fertilized”;
for instance, data obtained from local reports.

^bTreatments included CO₂ concentration (elevated vs. ambient) combined with two irrigation regimes (well irrigated vs. limit irrigated).

^cTreatments included sowing dates combining with varied temperatures.

Table 5 Summary of the CERES-Wheat model performances for maturity simulations.

Treatment category	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized only	Australia	RMSE: 2.1 days	Thaler et al. (2012)
Irrigated with a gradient of water/ different scheduling time and well fertilized only	Spain Mexico	15 days earlier ^a Difference: 7 days	Iglesias et al. (2000) Lobell and Ortiz- Monasterio (2006)
Well irrigated and fertilized with a gradient of fertilizer(s)	India	RMSE: 4.5 days, normalized RMSE 3.4%	Timsina et al. (2008)
Well irrigated and well fertilized ^b	Argentina, China	RMSE: <5 day	Caviglia et al. (2013); Liu and Tao (2013)
	United Kingdom, Czech Public	RMSE: 7.5 ~ 10 days	Bannayan et al. (2003); Trnka et al. (2004)
	China	Difference: <10 days	Liu and Yuan (2010); Xiao et al. (2013); Zhao et al. (2011); Tian et al. (2012); Wang et al. (2012); He et al. (2013)
	United Kingdom	R ² : 0.68	Cho et al. (2012)
	Spain Bangladesh	RMSE: 11 days RSME: 2.3 days	Abeledo et al. (2008) Timsina et al. (1998)
Not irrigated and fertilized with a gradient of fertilizer(s)	United States	4 days earlier ~ 1 day delay	Saseendran et al. (2004)
	Germany	Difference: >15 days	Bacsi and Zemankovics (1995)

CO ₂ concentration	United States	2 days delay Normalized RMSE: 4.3%	Tubiello et al. (1999a) Tubiello et al. (1999b)
Sowing Date	India	6 days earlier ~ 3 days delay	Hundal and PrabhjyotKaur (1997)
	United States, Algeria	RMSE: <5 days	White et al. (2011)^c , Rezzoug et al. (2008) ; Xue et al. (2004)
Spacing	United States	RMSE: 8 ~ 9 days	Xue et al. (2004)
	Pakistan	RMSE: 23 days	Sultana et al. (2009)
	United States	Difference: <10 days	Southworth et al. (2002)
	United States	Average error: 3 days	Tsvetsinskaya et al. (2003)

^aThe authors pointed out that the large difference was partly due to they counted harvest date as maturity date, instead of counting physiological maturity.

^bLiteratures that did not include treatments were considered as “well irrigated and well fertilized only”; for instance, data obtained from local reports.

^cTreatments included sowing dates combining with varied temperatures.

(Wang et al., 2009). The emergence dates were perfectly simulated for wheat grown in arid Northwest China under nine irrigation treatments of various amounts and timings (He et al., 2013). However, the calibrated CERES-Wheat model simulated the emergence date as being 7 days earlier for winter wheat grown on six irrigated sites in China (Liu and Yuan, 2010). A booting date simulation was 2 days delayed under elevated CO₂ concentration (roughly 450–500 ppmv) in the Intensive Agricultural Biome of Biosphere 2 (Tubiello et al., 1999a) and had an average RMSE of 5.3 days for three wheat cultivars sown in early- and mid-Oct. (Xue et al., 2004). Xue et al. (2004) also reported that the mean RMSE between the observed and simulated heading dates was 4.8 days. The simulated terminal spikelet date was 6 days earlier in one of the irrigated wheat production regions in China (Liu and Yuan, 2010) and 3 days earlier under varied, elevated CO₂ concentrations (Tubiello et al., 1999a). Nonetheless, terminal spikelet emergence was up to 19 days late for one wheat cultivar, but up to 15 days earlier for the other under different sowing date treatments (Porter et al., 1993). A similar study with different planting dates and three wheat cultivars showed an average RMSE for terminal spikelet simulation of 6.2 days (Xue et al., 2004). The reported ratios of simulated and observed terminal spikelet in dry-land Canada ranged from 0.64 to 1.62 (Chipanshi et al., 1997). The end of vegetative growth and the end of year growth were studied using fertilized winter wheat field data in China and Canada. The test results for China indicated that the simulations were 1 and 8 days different from the observed end of vegetative growth and end of year growth, respectively (Liu and Yuan, 2010), and for Canada, the ratios between the simulations and the observations were 0.67 ~ 1.44 and 0.67 ~ 1.43, respectively (Chipanshi et al., 1997). The duration of the vegetative period was tested in two production regions in the Czech Republic, yielding underestimated results by 3 or 4 days (St'astna et al., 2002). The simulated flowering dates in wheat production stations in China were correlated with the observations ($R^2 > 0.6$) and were within 7 days of the observations (Wang et al., 2012; Zhao et al., 2011). The anthesis date was extensively tested under a wide variety of treatments and locations. The differences between the simulated and the observed anthesis dates were within 10 days with an elevated CO₂ concentration (about 440 ppmv) treatment (Tubiello et al., 1999a); irrigated fields in Mexico (Lobell and Ortiz-Monasterio, 2006), fertilization treatments in two states of the United States, Colorado (with 0 ~ 112 kg N/ha application rates) (Saseendran et al., 2004) and Arizona (with 110.5 and 241.0 kg N/ha) (Thorp et al., 2010b); well-irrigated and fertilized experiments in Arizona, USA (Ottman et al., 2013); 15-year

simulations in Austria (Alexandrov et al., 2002); and nine irrigation treatments in China (He et al., 2013). On average, the simulated anthesis date matched the observations for three experimental stations in South Carolina (USA) between 1991 and 1995 (Tsvetsinskaya et al., 2003). The average errors regarding the simulated anthesis date were within 7% across planting dates in late-Sep. and mid-Nov. (Nov. 10 and 16) when combined with high N rates (213 ~ 232 kg/ha) versus no N application in Germany (Bacsi and Zemankovics, 1995) and three sowing dates in Dec. in Algeria (Rezzoug et al., 2008). The average RMSEs were within 8 days under different water availability treatments combined with various nutrient availability experiments in Bangladesh (Timsina et al., 1998) and Spain (Abeledo et al., 2008), four sites in the United Kingdom (Bannayan et al., 2003), rain-fed fields in Nebraska (Xue et al., 2004), no water or nutrient stress conditions in Argentina (Caviglia et al., 2013), rotation cropping systems with different irrigation schedules and nitrogen application rates in India (Timsina et al., 2008), five wheat production stations in the Czech Republic (Trnka et al., 2004), elevated temperature combined with various sowing dates (White et al., 2011), and dry areas in Northeastern Austria (Thaler et al., 2012). The simulated anthesis dates were within the normalized RMSE of 8% for three wheat varieties in Italy (Dettori et al., 2011) and under an elevated CO₂ concentration treatment (Tubiello et al., 1999b). Nonetheless, two studies reported less accurate anthesis simulations under well-irrigated and fertilized conditions in New Zealand and India, with RMSEs of up to 22 days (Porter et al., 1993) and up to 9 days of error (Hundal and PrabhjyotKaur, 1997), respectively. When applying the CERES-Wheat model to wheat production stations in China, the simulated anthesis dates were within 4.5% error for four of the stations (Liu and Tao, 2013) and less than 5 days for eight of the stations (Xiao et al., 2013). Using 36 wheat observation stations and 42 cropping zones in China, Tian et al. (2012) reported errors of 6.5% for winter wheat and of 12% for spring wheat (Tian et al., 2012). In contrast, Liu and Yuan (2010) reported that the simulated anthesis date was 9 days earlier for winter wheat in the Southern North China Plain. In addition, the root mean square error for the wheat anthesis date simulation in adequate water availability versus early drought experiments was reported to be 2.7 days in Argentina (Savin et al., 1994). For a grain filling simulation, the beginning of the grain filling date was 1 day earlier than the observation under elevated CO₂ concentration conditions (Tubiello et al., 1999a) and, on average, 4 days earlier for four wheat production stations in China (Liu and Yuan, 2010). Chipanshi et al. (1997) reported that the simulated to observed beginning of grain filling date ratios were from 0.77 to 1.41 over 30 years for a long-term

experiment site in Canada. In a heated environment with varying sowing dates, the simulated duration of grain filling showed less than 5 days RMSE (White et al., 2011). Wheat maturity date has been intensively tested under various treatments and locations as well. Under a variety of growing conditions, RMSEs for maturity simulation were less than 11 days. Those conditions and treatments included a combination of water stress (irrigated vs. rain-fed) and nutrient stress (with vs. without nitrogen application) in Spain (Abeledo et al., 2008); water- and nutrient-stress-free fields in Argentina (Caviglia et al., 2013); a heated environment (White et al., 2011); four locations over 3 years in the United Kingdom (Bannayan et al., 2003); five wheat production stations in the Czech Republic (Trnka et al., 2004); wheat rotated with rice or maize or soybean in various soils (Timsina et al., 2008); four wheat stations in China (Liu and Tao, 2013); six irrigated wheat production stations in the North China Plain (Liu and Yuan, 2010); nine wheat cultivars sown on three dates in Dec. (Rezzoug et al., 2008); eight planting dates ranging from Aug. 24 to Nov. 3 with seeding rates of 151, 301, 452, and 603 seeds/m² in Wisconsin (Dahlke et al., 1993; Southworth et al., 2002); eight winter wheats in the North China Plain (Xiao et al., 2013), early- and mid-Oct. sowing dates in fertilized plots in Nebraska (Xue et al., 2004); two locations Henan Plain in China (Zhao et al., 2011); winter wheat in dry regions in Austria for 9 years (Thaler et al., 2012); combinations of two water regimes (well-irrigated and rain-fed) and three nitrogen application regimes (0, 90, and 135 kg/ha) in Bangladesh (Timsina et al., 1998); and fertilized fields with various spacings in the Southeastern United States (Tsvetsinskaya et al., 2003). Studies in China showed a well-simulated maturity date. One study showed that in 36 production stations in China over 38 years, the relative absolute errors for both winter wheat and spring wheat were within 10 days (Tian et al., 2012), and two other studies showed that the errors were within 2 days in their studied regions (He et al., 2013; Wang et al., 2012). Under fluctuating and elevated CO₂ concentrations (about 440 ppmv) (Tubiello et al., 1999a), sowing dates ranging from DOY310 to DOY354 (Hundal and PrabhjyotKaur, 1997), and fertilization application treatments (0 ~ 112 kg N/ha) in rain-fed fields in the United States (Saseendran et al., 2004), the simulated maturity dates were within 7 days of error. Furthermore, the normalized RMSE was reported to be 4.3% for a maturity simulation performed with a 550 ppm CO₂ concentration treatment (Tubiello et al., 1999b). However, the differences between the simulation and the observation were over 15 days for both fertilized (with 213 ~ 232 kg N/ha rate) and unfertilized wheat maturity simulations in four sites in Germany (Bacsi

and Zemankovics, 1995), experimental trials in Pakistan (Sultana et al., 2009), and wheat production sites in Spain (Iglesias et al., 2000). Lobell and Ortiz-Monasterio (2006) indicated that in a no-water-stress situation, the CERES-Wheat simulated maturity date was exactly the same as the observed one, while in a water-stress situation, the difference between the simulation and the observation was up to 7 days. In addition, Cho et al. (2012) reported a high correlation ($R^2 = 0.68$) between the observed and simulated maturity dates. Monzon et al. (2007) reported that the RMSE for simulating both anthesis and maturity in multiple fields in Argentina was 4.9 days. The overall phenological stage was tested in six irrigated wheat production sites in China and with ambient versus elevated CO_2 concentrations combined with irrigation treatments (adequate vs. limited water supply); this resulted in an RMSE of 5.6 days (Liu and Yuan, 2010) and a normalized RMSE of up to 15% (Biernath et al., 2011), respectively. The phenological event simulations had RMSEs of 3 ~ 4 days given rice residue treatment (removed vs. remained) with irrigation treatment (Sarkar and Kar, 2008) and under various soils with fertilizer applications in Canada (He et al., 2014). Nonetheless, a study using 848 field datasets in Germany showed that the average difference between the simulated and observed period from terminal spikelet development to the end of leaf growth and the beginning of year growth could be as large as 9.1 days (Johnen et al., 2012).

3.1.3 CERES-Rice

The CERES-Rice model has been tested for heading (two studies), flowering (three studies), anthesis (seven studies), maturity (six studies), days from panicle initiation and grain filling to maturity (two studies), and phenological events (one study). Yun, (2003) tested the duration from transplanting to heading in two crop experiment stations in Korea and reported that the simulation was in good agreement with the observations, with an R^2 of 0.85. Zhang et al. (2013) reported that the RMSEs were within 5 days for a heading date simulation in single- and double-season rice zones in China. Rice flowering duration was tested with 32 field experiments combining various seeding and transplanting dates, planting densities, nitrogen fertilizer rates, and levels of irrigation across India and eight rice ecological stations in China, with reported RMSEs of 4.5 days (Mall and Aggarwal, 2002) and 5.5 days (Yao et al., 2007), respectively. The simulated days to flowering were within 5% error for four rice varieties under fertilization

treatments (0 ~ 150 kg N/ha rates and different timings) in Thailand (Cheyglinted et al., 2001). The anthesis date has been tested with five irrigation levels between 625 and 1225 mm and a seedling density between 1 and 3 seedlings per hill in Pakistan, and the simulated anthesis date was only 1 day earlier than the observed date (Ahmad et al., 2012). In contrast, the percentage error for the simulated anthesis dates given a late-Aug. seeding date treatment was 6.85% (Babel et al., 2011). The anthesis date has also been tested with water availability treatments combined with various nutrient availability experiments in Bangladesh (Timsina et al., 1998), six rice production areas in China (Tao et al., 2008), conventionally tilled soil with residual removal and direct-seeding, mulch-based cropping systems combined with manure, NPK¹, and dolomite fertilizer applications in Madagascar (Gerardeaux et al., 2011), with RMSEs ranging from 4.2 to 8.2 days. In addition, the differences between the simulated and observed days to anthesis under open field and elevated CO₂ treatments were between 2 and 4 days (Satapathy et al., 2014). The inter-annual variability of the rice anthesis date for three agricultural experiment locations in the Southern US was well-reproduced by the CERES-Rice model (Tsvetsinskaya et al., 2003). The simulated maturity dates matched perfectly with the observations under irrigation and planting density treatments in Pakistan (Ahmad et al., 2012). The reported RMSEs for maturity date simulations were within 10 days for tillage treatments in Madagascar (Gerardeaux et al., 2011), six rice production stations in China (Tao et al., 2008; Zhang and Tao, 2013), and irrigation combined with fertilization treatment in Bangladesh (Timsina et al., 1998). Over 500 experimental rice stations in China, the RMSE for maturity simulation ranged from 10 to 25 days (normalized RMSE: 6.5 ~ 19.8%) (Xiong et al., 2008b). The simulated days to the appearance of panicle initiation, flowering and maturity matched well with the observations given varying nitrogen application rates (0 ~ 150 kg/ha) in India, resulting in 3 ~ 5 days of errors (Swain and Yadav, 2009). The simulated period from grain filling to maturity was reasonably accurate and correlated with the observations ($r^2 = 0.72$) in Korea (Yun, 2003). Phenological events were tested with transplanted and direct-seeded rice with wheat residue and various nitrogen input treatments in rain-fed fields in India. The RMSEs for the phenological event simulations were 4 ~ 5 days and 10 ~ 11 days for transplanted and direct-seeded rice, respectively (Sarkar and Kar, 2008). A summary of the phenological variable validations for the CERES-Rice model can be found in Table 6.

¹NPK: 11% N, 22% P₂O₅, 16% K₂O (Gerardeaux et al., 2011).

Table 6 Summary of the CERES-Rice model performances for phenology variable simulations.

Treatment category	Variables	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized	Anthesis	Thailand	Percentage error: 6.85%	Babel et al. (2011)
Well irrigated and fertilized with a gradient of nitrogen inputs	Flowering Maturity	Thailand India, China	Percentage error: <5% Error: within 3 ~ 5 days RMSE: 10 ~ 25 days	Cheyglinted et al. (2001) Swain and Yadav (2009); Xiong et al. (2008b)
Well irrigated and well fertilized ^a	transplanting to heading Grain filling to maturity	Korea	R^2 : 0.85 R^2 : 0.72	Yun (2003)
	Heading date	China	RMSE: 5 days	Zhang and Tao (2013)
	Flowering duration	China	RMSE: 5.5 days	Yao et al. (2007)
	Anthesis	China	RMSE: 5.6 days	Tao et al. (2008)
	Maturity	China	RMSE: 2 ~ 6.6 days	Zhang and Tao (2013); Tao et al. (2008)
Irrigated with a gradient of water and fertilized with a gradient of nitrogen	Anthesis Maturity	Bangladesh Bangladesh	RMSE: 4.3 days RMSE: 2.3 days	Timsina et al. (1998) Timsina et al. (1998)
Over 80 treatments ^b	Flowering duration	India	RMSE: 4.5 days	Mall and Aggarwal (2002)
Fertilized, irrigated with varied amount of water and planted with varied densities	Anthesis Maturity	India India	Difference: 0 ~ 1 day Difference: 0 day	Ahmad et al. (2012) Ahmad et al. (2012)

(Continued)

Table 6 Summary of the CERES-Rice model performances for phenology variable simulations.—cont'd.

Treatment category	Variables	Countries	Performance	References
Management intensities ^c	Anthesis	Madagascar	RMSE: 8.2 days	Gerardeaux et al. (2011)
	Maturity	Madagascar	RMSE: 10.2 days	Gerardeaux et al. (2011)
Varied planting dates and nitrogen inputs	Anthesis	United States	Very accurate	Tsvetsinskaya et al. (2003)
Open field and elevated CO ₂	Anthesis	India	2 ~ 4 days	Satapathy et al. (2014)
	Maturity		3 ~ 11 days	Satapathy et al. (2014)
Direct seeded and transplanted rice	Phonological events	India	RMSE: 4 ~ 11 days	Sarkar and Kar (2008)

^aLiteratures that did not include treatments were considered as “well irrigated and well fertilized”; for instance, data obtained from local reports.

^bTreatments included varied seeding and transplanting dates, planting densities, spacing, nitrogen inputs and irrigations.

^cManagement practices included conventional tillage with residual removal and direct seeding mulch-based cropping system, and fertilized with manure, NPK and dolomite combinations.

3.2 Grain Yield

There were a total of 140 studies on the yield tests for the CERES models. The models have been tested under various experimental conditions, including well-managed fields with adequate irrigation and fertilization application and management treatments such as contrasting irrigation and fertilization application, varied sowing dates and population density, various planting methods, and various tillage methods and CO₂ concentrations. In general, the simulated grain yield matched reasonably well with the observed data, with RMSEs under 1400, 1200, and 800 kg/ha for maize, wheat, and rice yield simulations, respectively.

3.2.1 CERES-Maize

Eighty-four studies have validated CERES-Maize grain yield simulations under a range of conditions. Overall, the average RMSEs for grain yield simulations were mostly ranged between 200 and 1400 kg/ha.

With adequate nitrogen input (240 and 401 kg N/ha) treatments in Florida, US, the RMSE for the maize yield simulation ranged from 305.6 to 539.5 kg/ha (Lizaso et al., 2011). Under well-irrigated and fertilized conditions in Iowa, Louisiana, North Carolina, and Colorado (US), the average RMSEs for the yield simulation were about 1000 kg/ha (Saseendran et al., 2005; Thorp et al., 2007; Tsvetsinskaya et al., 2003; Yang et al., 2009). The average normalized RMSE for eight maize cultivars under irrigated and fertilized treatments in Iran was 3.55% (Moradi et al., 2014). In contrast, the reported RMSEs were from 1315 to 2194 kg/ha in Georgia (US), Spain, and in a wheat-soybean-maize rotation system in Argentina (Caviglia et al., 2013; López-Cedrón et al., 2005; Tsvetsinskaya et al., 2003). Nonetheless, Tsvetsinskaya et al. (2003) and López-Cedrón et al. (2005) reported that the errors were less than 4% for yield simulations in the United States. Basso et al. (2007) also reported RMSEs around 2000 kg/ha for a whole field, high-yield zones, and low-yield zones. The average RMSE for simulating five maize production stations in China was 1347.6 kg/ha, but the error was within 20% (Tao and Zhang, 2010). Liu (1989) also reported that the error for a 4-year maize yield simulation in Brazil ranged from 10 to 21%, depending on the soil water initialization. Epperson et al. (1993) showed that for an irrigated field, the simulated yield was not significantly different from the observed yield at a 1% significance level. The differences between simulated and observed maize yields were within 600 kg/ha for 2-year simulations in China and 30-year simulations in Nigeria (Jagtap and Abamu, 2003; Wang et al., 2012). Studies

in two locations in Zimbabwe and a sheltered intercropping system in the United States showed that the simulation errors were within 9% (Makadho, 1996; Mize et al., 2005). The simulations for six locations in Southern Québec, Canada were mostly within 7.7% for overestimation, but the error could be up to 82.2% for underestimation (Brassard and Singh, 2007). The simulated maize yield in experimental stations in Bulgaria was close to the measured yield (Alexandrov and Hoogenboom, 2000). Wang et al. (2011) and Ye et al. (2012) showed that the CERES models were able to capture spatial grain yield variation in China. Link et al. (2006) and Paz et al. (1999) also showed that the CERES model could explain 60% of spatial maize yield variability for over 5 years in Germany and 57% of temporal and spatial yield variability in a farm in the United States, respectively. On a regional scale, the simulated maize yield for over 30 years in a county in Indiana, US, was not significantly different from that in the report ($p = 0.05$) (Andresen et al., 2001). Heinemann et al. (2002) indicated that the grain yield simulation in Brazil was acceptable on a regional scale. The average root mean square deviations (RMSDs) for yield simulations at a county level for over 9 years in nine states in the United States ranged between 610 and 1520 kg/ha, with R^2 values from 0.05 to 0.8 (Kiniry et al., 1997). Weak correlations ($r \leq 0.7$) between the simulated and the recorded yield at the county level have been reported for Panama (Ruane et al., 2013) and the United States (Dhakhwa et al., 1997).

Studies have also tested grain yield under both nutrient and water stress conditions (ie, the treatment had contrasting irrigation and fertilization applications or had no nitrogen or no irrigation input). Examining four levels of nitrogen addition ranging between 20 and 280 kg/ha combined with two irrigation treatments involving fixed- versus variable-deficit trigger schedules, Pang et al. (1998) found that the simulated grain yields matched well with the observations for only the 20 and 100 kg N/ha treatments, not for the 180 and 280 kg N/ha treatments; the modeled grain yield was about 1.5 Mg/ha higher than the observed yield. Others, however, demonstrated that the CERES model was able to simulate grain yield accurately across a range of nutrient treatments (zero to high fertilizer input) combined with a range of irrigations, with an average RMSD under 360 kg/ha (Binder et al., 2008), average normalized RMSEs of 5.3% (Moradi et al., 2013), and an R^2 of 0.936 (Pang et al., 1997). Persson et al. (2009) showed that the simulation errors were 0.55 ~ 27.9% for irrigated and rain-fed maize with fertilization and planting date treatments. Sadler et al. (2000) indicated that under rain-fed conditions in South Carolina, US, with a nitrogen input of

0 ~ 220 kg/ha and a planting density of 0 ~ 15 plants/m², the simulated yield matched the measured yield reasonably well for some years, but not for other years.

The CERES models have also been tested given sufficient nutrients and varied available water conditions. The available soil water ranged between 30 and 75% of the maximum allowable depletion treatment. Panda et al. (2004) reported that the average RMSDs for wheat and maize grain yield simulation were under 250 kg/ha. With rain-feeding and 116 kg N/ha application treatments in Florida, the RMSE for the simulated maize yield was 290.8 kg/ha (Lizaso et al., 2011). Given irrigation treatments from optimum to moderate water stress in Pakistan, the RMSEs associated with the simulated yield for 2 years were under 560 kg/ha (Iqbal et al., 2011). Across six irrigation treatments involving 40 ~ 100% full irrigation in Colorado, US, the average normalized RMSE was under 10% (Anothai et al., 2013). Across irrigated and dry land in nine towns in Texas, US, and two irrigation treatments (with 421 and 609 mm irrigation) in Spain, the RMSEs ranged from 630 to 2140 kg/ha (Dechmi et al., 2010; Kiniry and Bockholt, 1998). Furthermore, across well-irrigated versus severe water shortage treatments in Australia and in a rain-fed treatment in Spain, the reported average RMSDs were over 3000 kg/ha (Carberry et al., 1989; López-Cedron et al., 2008). By comparison, across three water-availability treatments (no, moderate, and severe water stress), the percentage errors were within 24%, and the simulations were more accurate for no-water-stress treatment (<12% error) than for moderate and severe water-stress treatments (>15%) (Ben Nouna et al., 2000; Mastrorilli et al., 2003). A similar pattern has been reported for experiments regarding water-stress versus sufficiently irrigated treatments in Pakistan (Mubeen et al., 2013). The reported average percentage errors for simulated maize yield on irrigated and dry land in the Corn Belt of the United States were within 10% (Hodges et al., 1987; Xie et al., 2001). The grain yield simulation was better in irrigated land than in rain-fed land for four maize hybrids in Brazil as well. The average normalized RMSDs were 3.78 and 8.29% for irrigated and rain-fed maize, respectively (Soler et al., 2007). Interestingly, another study of five rain-fed maize production stations in the North China Plain found that the model underestimated maize yield in dry years with percentage errors of 11 ~ 64% and overestimated the yield in wet years with percentage errors of 26 ~ 55.4% (Wu et al., 1989). Under rain-fed conditions, three studies reported reasonably accurate yield simulations, with errors of 5 kg/ha for 1 year and 357 kg/ha for the other year in Nigeria (Jagtap et al., 1993), an underestimation

within 10% in Croatia (Vucetic, 2011), and good matching between simulated and observed yields for three sites in Venezuela (Maytin et al., 1995). For a rain-fed maize yield simulation on a regional scale in China, the reported RMSD was 1898 kg/ha, and on a farm scale, the simulated yield was highly correlated ($R^2 = 0.96$) with the observations in four rain-fed maize production stations (Xiong et al., 2007). For rain-fed fields in the Czech Republic, the simulated yield was within 17% error, and ignoring simulations with unusual weather, the simulations were within 12% error (Žalud and Dubrovský, 2002). In rotational rain-fed fields in Canada with tile drainage versus controlled tile drainage-subsurface irrigation treatments, the model efficiencies were 0.987 and 0.998, respectively, and the average normalized RMSDs were 14 and 4.3%, respectively (Liu et al., 2011). Tubiello et al. (2002) indicated that the simulated maize yield matched reasonably well with the observed yield under rain-fed conditions in five US states. Saseendran et al. (2008) tested the model using 3 years of grain yield measurements in Colorado, US, under both line-source sprinkler irrigation (with 23 ~ 106 mm, 72 ~ 188 mm, and 46 ~ 299 mm water) and rain-fed treatments. The authors reported that the simulated RMSDs were 982 and 576 kg/ha for irrigation and rain-fed treatments, respectively. DeJonge et al. (2012) also reported that the model simulated fully irrigated grain yield more accurately than limit-irrigation grain yield, with 2.47% relative error for the full-irrigation yield simulation and 12.90% for the limit-irrigation yield simulation. By contrast, DeJonge et al. (2011) simulated grain yield under adequate water and limited water treatments on the same experimental location and reported relative errors of 4.1 and 3.4% for full irrigation and limited irrigation, respectively. The grain yield simulation for an experiment with 100%-, 75%-, and 50%-full irrigation treatments in Turkey also showed that the model underestimated grain yield by 4.9, 1.7, and 9.4%, respectively, 1 year and by 4.6, 3.8 and 2.3%, respectively, another year (Gercek and Okant, 2010). The reported mean errors for simulated grain yields at three sites for 3 years were 850, 933.3, and 333 kg/ha for 50%-, 75%-, and 100%-full irrigation treatments, respectively (Dogan et al., 2006). In the same research, the model was tested for overirrigated treatments as well. Given 65%-, 100%-, and 135%-full irrigated treatments for 3 years, the mean errors associated with the simulated yields were 1400, 600, and 3250 kg/ha, respectively (Dogan et al., 2006). One study in Spain considered wind speed's effect on irrigation and compared the grain yield simulations for nighttime irrigation and daytime irrigation (Salmerón et al., 2012). The reported RMSDs for the night irrigation treatment were around 1000 kg/ha

for 3 years, while the RMSDs for the daytime irrigation treatment were over 1200 kg/ha for 2 years and 935 kg/ha for another year (Salmerón et al., 2012). Additionally, the simulated maize yield was highly correlated ($R^2 = 0.66$) with the reported provincial yield under primary rain-fed conditions in South Africa (Estes et al., 2013).

With fully irrigated but varying nutrient applications between 0 and 140 kg/ha, the average RMSDs in both summer and spring maize yield simulations were 350 kg/ha lower in China (Binder et al., 2008). In Florida, US, without nitrogen fertilizer application, the RMSE for the simulated grain yield was 116.7 kg/ha (Lizaso et al., 2011). When three nitrogen input rate (300 ~ 400 kg N/ha) and two sowing date (Oct. 15 and Nov. 15) treatments were applied to two maize cultivars in Chile, the average RMSE of the simulated yield was 691 kg/ha (Meza et al., 2008). Two model tests with nitrogen treatments of 0 ~ 150 kg/ha and 0 ~ 250 kg/ha in Thailand and Hungary, respectively, indicated that the simulated grain yields were close to the observations, except for those without the nitrogen addition treatment (Asadi and Clemente, 2003; Kovacs et al., 1995). Miao et al. (2006) also showed that with fertilization treatments (112 ~ 336 kg N/ha applications), the simulation errors were mostly within 10%, while in a no-fertilization treatment, the absolute errors were over 20%. In China, maize yield simulations given 0 and 165 kg N/ha application rates had normalized RMSEs of 32 and 23%, respectively (Yang et al., 2013). Similarly, in Canada, while the normalized RMSE for continuous maize with fertilization application treatment was 6%, the normalized RMSE for maize without fertilization application was 37% (Liu et al., 2014). In Ethiopia, with fertilizer addition treatments between 0 and 100 kg/ha, the model overestimated maize yield by about 300 kg/ha (Kassie et al., 2014). A study using 50 years of experimental data reported that the grain yield simulations for maize in fertilized (side-dress nitrogen of 112 kg/ha) versus nonfertilized fields showed a much higher normalized RMSD in the non-fertilized field (82%) than in the fertilized field (39%) (Liu et al., 2010). O'Neal et al. (2002) pointed out that the low correlation ($R^2 = 0.33$) between the observed and simulated grain yields given 127 ~ 227 kg N/ha application treatments was due to lower levels of fertilizer application in the treatments used. The normalized RMSDs found in the maize grain yield simulation across 0 ~ 400 kg/ha nitrogen application treatments in China were small as well (within 15%) (Liu et al., 2012). In Thailand, using a treatment with mineral fertilizer application rates and four compost fertilizer rates (0 ~ 7500 kg/ha), the grain yield was relatively well-simulated in the

second and third years (normalized RMSE of 11.1 ~ 16.9% and an index of agreement over 0.55) but poorly simulated in the fourth and fifth years (normalized RMSE of 62.8 ~ 107.0% and an index of agreement below 0.45) (Pinitpaiboon et al., 2011). A study in Burkina Faso simulating grain yields given no, inorganic, and organic nitrogen inputs showed that the simulated yield was 667 kg/ha higher than the observed yield (Soler et al., 2011). In addition, with fertilization rates of 60 ~ 120 kg N/ha, the simulated yields were lower at 60 kg N/ha but higher at 120 kg N/ha compared to the observed yield (Jagtap et al., 1999).

Other test conditions included various sowing dates, planting densities, tillage methods, planting methods, soil types, CO₂ concentrations, leaf defoliation, and winter cover crops. Simulating various hybrid maize yields using four sowing dates in India (Jun. with 10-day intervals between sowing dates) and Argentina (Aug. 20 ~ Nov.), the average RMSDs were 559 and 3670 kg/ha, respectively (Otegui et al., 1996; Ramawat et al., 2012). In the historical field data for sowing date treatments in Illinois, US, the simulated yield reflected the inter-annual yield variation, and the errors were within 10% (Southworth et al., 2000). In Iran, a validation study was conducted with three maize cultivars and seven planting densities (3, 5, 7, 9, 11, 13, and 15 plants/m²), and it showed that the average normalized RMSD was within 9% (Lashkari et al., 2011). However, a study simulating maize yield with 0 ~ 24 plants/m² density at two sites in North America showed that the model did not capture yield reduction in response to the increased planting density at both sites (Ritchie and Alagarwamy, 2003). The reported normalized RMSDs for simulated yield in China were 9, 16, and 23% for conventional tillage, reduced conventional tillage, and no tillage, respectively (Liu et al., 2013). Hook (1994) reported that the grain yield in four types of soil in Georgia, US, was overestimated by 260 kg/ha on average and that the error ranged between an underestimation of 1680 kg/ha and an overestimation of 1120 kg/ha. The grain yield simulation was also tested under nutrient stress combined with tillage method treatments. One research project used 4-year datasets from maize fields under conventional, rational, and dish harrow tillage with various fertilizer applications (including calcium chloride, single super-phosphate, and calcium ammonium nitrate) and calculated the mean absolute errors as 547, 645, and 1030 kg/ha, respectively, for each tillage treatment (Samuhel and Siska, 2007). With a 66% leaf defoliation rate at leaf stages 6 and 12, the CERES-Maize model yield prediction was less than desirable, with absolute percentage errors of 14 ~ 34% (Weiss and Piper, 1992). In general, the simulated maize grain yield matched well with the

observed maize yield across no-cover crop treatments and winter cover crop (barley, oilseed rape, winter rape, and vetch) treatments in Spain, with RMSEs ranging from 530 to 2720 kg/ha (Salmerón et al., 2014).

3.2.2 CERES-Wheat

The model has been validated under a range of conditions, such as irrigation, fertilization, sowing date, and tillage treatments.

Under irrigated and fertilized conditions, the simulated wheat yield accurately depicted the wheat yield trends for several rice-wheat sites in India (Subash and Mohan, 2012). Several studies of wheat production showed that the simulated grain yield matched the observed yield well, with low RMSEs (RMSE: 175 ~ 588.6 kg/ha), low errors (error: <500 kg/ha), and high correlations of determination (R^2 : 0.9) (Liu and Tao, 2013; Liu and Yuan, 2010; Wang et al., 2012; Zhang et al., 2013; Zhao et al., 2011). Nonetheless, a large RMSE of 897 kg/ha was reported when comparing the simulated grain yield for 141 wheat stations in China and the consensus yield (Xiong et al., 2008a). In general, the model accurately simulated wheat yields for fertilized wheat fields in Italy (RMSEs: <950 kg/ha) (Basso et al., 2007, 2009), two sites in Canada over 30 years (R^2 : 0.7) (Chipanshi et al., 1999), the Pampas (RMSE: 410 kg/ha, R^2 : 0.86) (Savin et al., 1995), wheat production stations in Bulgaria (the observations and simulations were scattered around a 1:1 line) (Alexandrov and Hoogenboom, 2000), four sites in the United Kingdom and five stations in the Czech Republic (RMSE: \leq 930 kg/ha, percentage error: 8.7%) (Bannayan et al., 2003; Trnka et al., 2004), and a site in Austria over 15 years (percentage error: <17%) (Alexandrov et al., 2002). The simulated grain yield was moderately well-matched with the observed yield for three durum wheat cultivars in Italy (average normalized RMSE of 27%) (Dettori et al., 2011), eight sites in Canada over 30 years (percentage error: <28%, with one exception of 58.4%) (Brassard and Singh, 2007), and eight sites in Europe (RMSE: 1603 kg/ha, agreement index: 0.74) (Palosuo et al., 2011). In general, the simulated wheat grain yield for four counties in the United States over more than 4 years matched well with the observations, with R^2 values ranging between 0.4 and 0.72 (Rosenzweig and Tubiello, 1996). In India, the modeled irrigated wheat grain yield was significantly different from the observed yield, but the yearly yield fluctuation was well-simulated (Lal et al., 1998). Tian et al. (2012) reported that the percentage error in grain yield simulation for 36 wheat production stations in China ranged from 11.6 to 33.6%, depending on the calibration methods.

Across available soil water from 10 to 60%, combined with four ratios of N:P₂O₅:K₂O fertilizer application (0, 80:40:40, 120:60:60, and 160:80:80) treatments, the average RMSDs were under 250 kg/ha, with a model efficiency of 0.95 (Behera and Panda, 2009). When simulating grain yield under both fertilizer application and irrigation treatments (five levels of nitrogen addition of 0 ~ 150 kg N/ha and irrigation 2 ~ 4 times per growing season), the CERES model performed the worst (absolute percentage deviation of 66.45%) when the crop was under the extreme conditions of low irrigation and no fertilization application, while it performed much better (absolute percentage deviation ranging between 0.38 and 12.9%) when the crop was at least fertilized with over 60 kg/ha nitrogen (Singh et al., 2008). Polilaities and Lazauskas (2010) simulated wheat grain yield in two experiments, one with 60 kg N/ha applied at various growing stages, and the other with various management intensities, and they concluded that the CERES model accurately simulated grain yield given 60 kg N/ha fertilizer addition treatment in the years without water shortage but that the accuracy declined when there was a water shortage or the plot did not receive nitrogen. In China, across treatments and with various levels of fertilization and irrigation, the normalized RMSE for grain yield was 5% (Ji et al., 2014). With fertilizer treatment and extreme conditions of both drought and nutrient deficit, the bias errors were 0.31, 0, and 0.74 for nonfertilized, fertilized at 45 kg N/ha, and extreme conditions, respectively (Touré et al., 1995). In experiments in Bangladesh with both nutrient (0 ~ 135 kg N/ha) and water treatments (rain-fed vs. irrigated), Timsina et al. (1998) reported that the overall simulated wheat yield matched well with the observations (RMSD = 467 kg/ha, $r^2 = 0.95$). Compiling 20-year yield data for a maize-wheat rotation given 0 ~ 250 kg N/ha input treatments, the maize and the wheat yields matched with the observations well (R^2 : 0.82) (Kovacs et al., 1995). In contrast, another study in Spain with similar water and nutrient treatments (rain-fed vs. irrigated and fertilized at 0 vs. 250 kg N/ha) showed that the wheat grain yield simulation had an average RMSD of 1060 kg/ha and that ignoring 1 year of rain-fed data, the average RMSD would have been reduced to 790 kg/ha (Abeledo et al., 2008). Similarly, simulating grain yield in an experimental field in India, combining five irrigation treatments of various amounts and timings and four nitrogen application levels from 0 to 180 kg/ha, resulted in an average normalized RMSD of 25%, but this would decrease to 18% if grain yield under extreme irrigation and nutrient treatment were not included (Arora et al., 2007). However, Lobell and Ortiz-Monasterio (2006) indicated that the CERES

model was able to simulate grain yield accurately across ranges of nutrient (low to high levels of nitrogen application) combined with a gradient of water applications (irrigation 3 ~ 5 times), with an average RMSD of 230 kg/ha. [Saseendran et al. \(2004\)](#) indicated that in rain-fed fields in Colorado, US, with fertilization treatment (0 ~ 112 kg/ha), the simulation was more accurate in years in which rainfall was plentiful.

Without nutrient stress but with available soil water ranging between 30 and 75% of the maximum allowable depletion treatment, [Panda et al. \(2003\)](#) reported that the average RMSDs for a wheat and maize grain yield simulation were under 250 kg/ha. Under rain-fed conditions in India, the CERES model simulated wheat yield reasonably well, with index of agreement of 0.87 and a model efficiency of 0.57 ([Vashisht et al., 2013](#)). For 13 US states, most of the wheat yield simulations were in good agreement with the recorded yield ([Tubiello et al., 2002](#)). [Iglesias et al. \(2000\)](#) found that the CERES model, in general, overestimated the grain yield across seven sites in Spain and that the simulation was more accurate for fully irrigated wheat than dry-land wheat. With a wheat yield simulation in Australia over 9 years, a study showed that the model overestimated yields with high values and underestimated yields with relatively low values, with an average error of 5.1%, and that the error was partly due to water stress ([Eitzinger et al., 2003](#)). The simulated grain yield across well-irrigated and early drought wheat fields in Argentina had a root mean square of 20 kg/ha, while the observed grain yield had a mean standard error of 40 kg/ha ([Savin et al., 1994](#)). Two studies compared the CERES model grain yield simulation's accuracy between fully available water and limited available water scenarios. [Singh et al. \(2008\)](#) calculated the RMSDs for grain yield simulations given irrigation 2 ~ 4 times per growing season and varied levels of nitrogen input for each irrigation treatment, and they reported that the RMSDs were over 700 and 220 kg/ha for the two least-irrigated treatments and the most frequent irrigation treatment, respectively. The errors for simulating wheat yield under irrigation 0, 1, 2, and 4 times in China ranged from a 390 kg/ha underestimation to a 150 kg/ha overestimation ([Yang et al., 2006b](#)). When simulating yield under nine treatments of various irrigation timings and amounts in China, the percentage errors ranged from 1.56 to 8.17% ([He et al., 2013](#)). Although accurate grain simulations under water stress have been reported, more studies found that grain yield simulation was not that accurate regarding fully irrigated conditions versus droughts in various growing stages treatments in New Zealand, with an RMSE of over 3000 ([Jamieson et al., 1998](#)). Interestingly, a test conducted in rain-fed

experiments in Syria and Italy, involving two wheat cultivars for 2 years showed percentage errors that were mostly over 60% and not more than 21%, respectively (Pecetti and Hollington, 1997). When simulating grain yield under limited versus full water availability combined with elevated versus ambient CO₂ concentration treatments in Germany, the model efficiency was 0.5 (Biernath et al., 2011). While simulated grain yield matched well with reported yield for irrigated and rain-fed wheat in Jordan (RMSE was 586 kg/ha), the simulated yield was weakly correlated with the observed wheat yield at the county level in Canada (correlation between two standardized anomalies was 0.59), Kansas (US) (R: 0.63), and two irrigated and fertilized fields in Mexico (R: 0.72 ~ 0.87) (Al-Bakri et al., 2011; Greene and Maxwell, 2007; Lobell et al., 2005; Mearns et al., 1992). Nonetheless, the model under-predicted yields for 303 wheat fields in England, with an average error of 2600 kg/ha (Landau et al., 1998). Nain et al. (2004) also reported a low correlation (R: 0.67) between the observed and simulated yield deviations (from the average yields) for three locations in India.

The CERES-wheat model has been tested using contrasting fertilization treatments and sufficient irrigation. The average RMSEs for the yield simulations were not larger than 471 kg/ha across various amounts and types of fertilizer application treatments in Pakistan (Bakhsh et al., 2013) and across five levels of nitrogen input with residue treatment in India (Sarkar and Kar, 2008). A study in India showed that a grain yield simulation across rotation cropping systems, with a 0 ~ 160 kg N/ha application being given to one of the systems, had an average RMSD of 617 kg/ha (Timsina et al., 2008). Wang et al. (2010) showed that for fertilization, irrigation and tillage treatments, the correlation coefficient between simulation and observation was about 0.95 for continuous wheat, while that for wheat rotation field was under 0.85. Zhang et al. (2012) suggested that with a 0 ~ 112 kg N/ha application to a wheat field, the simulated and observed grain yields were not significantly different ($P = 0.05$). Given 110.5 and 241.0 kg N/ha application treatments in Arizona, US, the average RMSE for the yield simulation was 7.4% (Thorp et al., 2010b). For six nitrogen input and planting density treatments in Arizona, the yield simulations were acceptable, with errors less than 1 Mg/ha (Thorp et al., 2010a). St'astna et al. (2002) found that for ten treatments, including fertilization rates (40 and 120 kg/ha), different crops preceding the wheat, and planting dates treatments, the CERES model could simulate grain yield relatively well when the crop was not infested by pest. Examining 30 years of field data regarding fertilization and non-fertilization treatments, Moulin and Beckie (1993) reported that the 1:1 line

between the simulation and the observation fell out of 95% confidence band, although there was a significant relationship between the simulated and the observed yield.

Other test conditions included various sowing dates, planting densities, tillage methods, planting methods, soil types, and CO₂ concentrations. A study in India in which wheat was sown between DOY310 and DOY354 showed that the model underestimated yield for both early- and late-sown wheat, with an average percentage error of 21% (Hundal and PrabhjyotKaur, 1997). Studies in Argentina and Pakistan showed that the model simulated wheat grain yield under varied sowing dates reasonably well, with RMSEs under 851 kg/ha (Monzon et al., 2007; Sultana et al., 2009). With experiments consisting of 31 sowing dates across Nebraska, US, for 6 years, the yield simulations for two wheat cultivars had average normalized RMSEs of 39 ~ 46% and RMSEs of about 1178 ~ 1266 kg/ha (Moreno-Sotomayor and Weiss, 2004). With both humid and dry weather in Algeria and using three sowing dates in Dec., the RMSE for simulating grain yield was 790 kg/ha (Rezzoug et al., 2008). For simulating grain yield using sowing dates between late-Aug. and early-Nov. combined with seeding rates from 14 to 56 seeds/ft.², the simulation accuracy varied greatly, with about 500 kg/ha overestimation for early sown treatment (before DOY247); good estimation for DOY255, 266, and 276; and up to 1600 kg/ha overestimation for late-sown treatments (sown after DOY284) (Dahlke et al., 1993; Southworth et al., 2002). Two studies tested the CERES model under a planting density treatment. Across seeding density (350 ~ 400 seeds/m²) combined with sowing date (Sep. 20 ~ Oct. 30) treatments, the average RMSD for the yield simulation was 240 kg/ha (Ghaffari et al., 2001, 2002). Across five planting density treatments, two irrigation levels, various levels of phosphorous input, and various seeding rates in Iran, the normalized RMSE was 5% (Bannayan et al., 2014). Under a wide range of growing conditions and various fertilizer inputs, sowing dates, and planting densities in five US states, the model tended to overestimate the grain yield (up to 36%), and the average overestimation was 8% (Tsvetsinskaya et al., 2003). Cho et al. (2012) reported that the CERES-Wheat model consistently overestimated the wheat yield in experiment fields given fertilization (48 ~ 192 kg/ha), sowing date (Sep. ~ Nov.), and seeding rate (350 ~ 450 seeds/m²) treatments in the United Kingdom for 11 years, with an r^2 of 0.56. With various rotation systems, Staggenborg and Vanderlip (2005) reported up to 22% grain yield overestimation with RMSEs of about 1400 kg/ha for both wheat-sorghum-fallow and wheat-fallow systems in Kansas, US. Given a range of tillage

treatments (eg, conventional tillage, surface tillage, minimum tillage, and no tillage), the model simulated grain yield in Spain (RMSEs: 551 kg/ha for conventional tillage and 804 kg/ha for no tillage) and Italy well (errors: 700 ~ 1200 kg/ha) (Castrignano et al., 1997; Soldevilla-Martinez et al., 2013). Langensiepen et al. (2008) noted that depending on the calibration process, the RMSE for wheat grain yield simulation in a wheat-barley-canola rotation field in Germany ranged from 700 to 2200 kg/ha. The grain yield prediction accuracy was reported for yields from various soil types as well. Eizinger et al. (2004) showed that the CERES-Wheat model overestimated the yield by 500 and 900 kg/ha with chernozem and sandy chernozem soils, respectively, and underestimated the yield by 1500 kg/ha with fluvisol soil. He et al. (2014) reported that the RMSEs for wheat yield simulation were under 1688 kg/ha across silt and clay soil sites in Canada. The model has been tested under ambient versus elevated CO₂ concentrations (550 ppm) in combination with well-watered versus water-deficit treatments, and the normalized RMSEs ranged from 9.2 to 23.3% (Tubiello et al., 1999b).

3.2.3 CERES-Rice

Tests regarding the rice yield of the CERES model are relatively less extensive but have still been performed using a range of treatments. The reported RMSEs mostly ranged from 200 to 1672 kg/ha.

Two studies in China simulating rice yield for six to eight rice ecological stations reported that the average RMSEs for rice yield were within 800 kg/ha (Tao et al., 2008; Yao et al., 2007). The simulated rice yield was not significantly different from the observed yield over more than 10 years of field experiments in two locations in India ($P > 0.01$) (Lal et al., 1998). Subash and Mohan (2012) showed that the CERES-Rice model predicted the rice production trends in several production sites for 29 years, although the model tended to overpredict the yield. By contrast, Xiong et al. (2008) reported that the CERES models failed to reproduce the temporal rice yield variability in China. Xiong et al. (2008b) also reported that the RMSEs for rice yield simulations for more than 500 rice stations in China ranged from 1129 to 1672 kg/ha (normalized RMSE: 11.8 ~ 25.6%) and that the model, on average, overestimated rice yield by 3191 kg/ha at a regional level. Yun (2003) showed that the correlation of determination between the simulated and observed yields was 0.4, while it was over 0.9 at the regional level when growing acreages were corrected.

Studies have tested grain yield under both water and nutrient stress conditions. Experiments in Bangladesh with both nutrient (0 ~ 135 kg N/ha)

and water treatments (rain-fed vs. irrigated), [Timsina et al. \(1998\)](#) showed that the overall simulated rice yield did not match well with the observations (RMSD = 1279 kg/ha, $r^2 = 0.52$). In contrast, [Amiri et al. \(2013\)](#) reported that the average RMSD was 297 kg/ha under various amounts of irrigations treatment and fertilization treatment (0 ~ 75 kg/ha) in Iran. Using 80 treatments consisting of various sowing dates, population densities, spacings, and fertilization and irrigation treatments, [Mall et al. \(2002\)](#) reported an overall RMSD of 698 kg/ha, but the model did not simulate grain yields accurately when the yield was under 4 Mg/ha ([Mall and Aggarwal, 2002](#)). Under rain-fed conditions in India, with six nitrogen application treatments (0 ~ 120 kg/ha) and wheat residue treatments (remains vs. removed), the simulated rice yield had a mean bias errors of 131.5 and 64.0 kg/ha for transplanted and direct-seeded rice, respectively ([Sarkar and Kar, 2008](#)).

The CERES models have also been tested under varied available water conditions. Combining irrigation water treatment (625, 775, 925, 1075, and 1225 mm) and seedling density (1 ~ 3 seedlings/hill) treatment in Pakistan, the simulated grain yield was on average 11% off of the observation, with a normalized RMSD of 1.4 ~ 2.1% for each seedling density treatment ([Ahmad et al., 2012](#)). Under rain-fed conditions with varying transplanting dates from mid-Jun. to early-Aug. in Bangladesh, the average RMSE for rice yield was 1270 kg/ha ([Mahmood et al., 2003](#)). [Godwin et al. \(1994\)](#) indicated that the CERES models performed less accurately when rice was under stressed conditions, with satisfactory yield prediction for continuously flooded and fertilized rice fields, heavy underestimation for continuously flooded and unfertilized rice fields, and heavy overestimation for sprinkled and fertilized rice fields.

With contrasting fertilizer input treatments, the CERES-rice model showed the ability to accurately simulate the rice yield as well. Simulating grain yield under a 0 ~ 150 kg N/ha treatment in India for a medium-duration rice variety showed the worst prediction among 150 kg/ha nitrogen addition treatments, with over 50% error, but the simulation error for the long-duration variety was 4 and 14% for the 150 kg N/ha and 10 kg N/ha treatments, respectively ([Swain and Yadav, 2009](#)). With the same fertilization treatment in three locations in Thailand, the biases were 277 ~ 407 kg/ha, while with a 75 kg N/ha addition, the biases were only 77 ~ 142 kg/ha ([Cheyglinted et al., 2001](#)). In addition, [Amien et al. \(1999\)](#) showed that the model slightly underestimated rice yield and that the simulated yield was highly correlated with the measured yield (r^2 of 0.87) in three regions of Indonesia using various nitrogen fertilizer sources, rates and application methods.

Others tested the CERES-Rice model using sowing date, tillage method, residue removal, elevated CO₂, and elevated temperature treatments. Babel et al. (2011) reported that the average percentage error for simulating both early- and late-sown rice in a field in Thailand was 3.13%. With 18 planting dates ranging from Jan. to Dec. and Jul. to Aug., in India, the yield simulations had indices of agreement ranging from 0 to 0.98 (the overall agreement index was 0.57) (Sudharsan et al., 2013). In Madagascar, the model was tested across two tillage treatments, conventional tillage with residue removal versus direct seeding with mulch-based tillage, and the average RMSD for the harvest weight simulation was 499 kg/ha (Gerardeaux et al., 2011). Sarkar and Kar (2006) reported that when simulating rice yield in rice-wheat rotation systems involving both residue removal and residue remaining, the average RMSDs were 267.34 and 445.21 kg/ha for transplanted rice and direct-seeded rice, respectively. Using an elevated CO₂ treatment in India, the grain yield simulation errors were within 10% (Satapathy et al., 2014). Across temperature treatments (0 ~ 2 °C elevated) combined with CO₂ concentration treatments (380 ppm and 550 ppm), the simulated yield was up to 17.6% underestimated and 9.1% overestimated (Kim et al., 2013).

3.3 Kernel Weight

3.3.1 CERES-Maize

Kernel weight has been tested on the basis of individual kernels, per square meter, and per year given irrigation, fertilization, and cover-crop treatments (Table 7). Six studies tested maize kernel weight under an irrigation treatment. Under water-stressed conditions, the kernel weight was underestimated by about 30%, while under irrigation treatments, the errors were about 3% (Mubeen et al., 2013). Across dry- and irrigated land in Kansas, US, the average RMSE for maize kernel weight was 0.061 g (Retta et al., 1991). Similarly, across well-irrigated and severe water stress treatments in Australia, the average RMSE was 0.13 g (Carberry et al., 1989). In Italy, the kernel weight tended to be underestimated, and the percentage errors were larger with severe water stress (up to 21%) than with moderate or zero water stress (within 15%) (Ben Nouna et al., 2000; Mastroianni et al., 2003). A study in Brazil indicated that the prediction of kernel weight was worse under rain-fed conditions (normalized RMSE of 6.07%) than under irrigated conditions (normalized RMSE of 4.8%) (Soler et al., 2007). When simulating grain weight in rain-fed fields in Nigeria, the errors were less than

Table 7 Summary of the CERES-Maize model performances for kernel weight^a and kernel number^a variable simulations.

Treatment category	Variables	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized	Kernel number	Brazil	RMSE: 102 ~ 257 Normalized RMSE: 4.56 ~ 12.87%	Soler et al. (2007)
		Nigeria	Difference: 0 ~ 174	Jagtap et al. (1993)
	Kernel weight	Spain	RMSE: 1700	López-Cedrón et al. (2008)
		Croatia	Underestimated by 3%	Vucetic (2011)
		Pakistan, Italy, China	Underestimated by 11 ~ 30%	Mubeen et al. (2013); Mastrorilli et al. (2003); Ben Nouna et al. (2000); Guo et al. (2010)
		Croatia	Overestimated by 39%	Vucetic (2011)
		Brazil	Normalized RMSE: <6.07%	Soler et al. (2007)
		Nigeria	Difference: <0.007 g Percentage error: <3%	Jagtap et al. (1993)
Irrigated with a gradient of water/different scheduling time and well fertilized	Kernel number	Italy	Underestimated by 1.7 ~ 21%	Ben Nouna et al. (2000); Mastrorilli et al. (2003)
	Kernel weight	Australia	RMSE: 1065	Carberry et al. (1989)
		United States	RMSE: 0.061 g	Retta et al. (1991)
		Australia	RMSE: 0.13 g	Carberry et al. (1989)
		Italy	Percentage error: <15%	Mastrorilli et al. (2003); Ben Nouna et al. (2000)

(Continued)

Table 7 Summary of the CERES-Maize model performances for kernel weight^a and kernel number^a variable simulations.—cont'd.

Treatment category	Variables	Countries	Performance	References
Well irrigated and well fertilized (Weiss and Moreno-Sotomayer, 2006)	Kernel number	Brazil	RMSE: 102 Normalized RMSE: 4.56%	Soler et al. (2007)
		Italy	Underestimated by: 8%	Ben Nouna et al. (2000); Mastrorilli et al. (2003)
	Kernel weight	Pakistan, Italy, Brazil	Percentage error: 3 ~ 15%	Mubeen et al. (2013); Mastrorilli et al. (2003); Ben Nouna et al. (2000); Soler et al. (2007)
	Kernel weight	Argentina	Overestimated it at its low weight range values, underestimated it at its high range values	Otegui et al. (1996)
Cover crops	Kernel number	Spain	RMSE: 188 ~ 584	Salmerón et al. (2014)
	Kernel weight		RMSE: 0.012 ~ 0.03 g	

^aHere only showed individual kernel weight and kernel number per square meter validation results; other relevant validated variables included kernel weight per square meter, kernel weight per year, kernel number per year and kernel number per plant and the results were summarized in the text.

0.007 g (within 3% error) (Jagtap et al., 1993). Under rain-fed conditions in Croatia, the model overestimated kernel weight by 39% (Vucetic, 2011). With cover-crop treatments in Spain, the kernel weight was well-simulated, with low RMSEs between 0.012 and 0.03 g (Salmerón et al., 2014). Grain weight per square meter was tested for dry- and irrigated land in Kansas, and the average RMSE was 152.2 g (Retta et al., 1991). The grain weight per year was tested for agricultural experiment stations in the Southeastern US, and the average RMSE was 59 g (Tsvetsinskaya et al., 2003). In addition, Otegui et al. (1996) indicated that when simulating kernel weight given sowing dates treatments for four maize hybrids in Argentina, the model overestimated the grain weight when it was at the low-range value but underestimated the grain weight when it was at the high-range value.

3.3.2 CERES-Wheat

Ten studies tested the weight of wheat kernels under a range of treatments (Table 8). The individual-grain-weight simulation had average RMSEs within 0.0076 g under water stress (rain-fed vs. irrigated treatments) combined with nutrient stress conditions (0 ~ 250 kg N/ha) in Spain (Abeledo et al., 2008) and fertilized cropping systems in Argentina (Monzon et al., 2007). Under well-irrigated and fertilized condition in India, the simulated grain weight for 5 years was 88 ~ 113% of the observed grain weight (Hundal and PrabhjyotKaur, 1997). In China, across nine irrigation treatments, the relative absolute errors ranged from 0 to 8.29%, with an average relative absolute error of 5% (He et al., 2013). Across rain-fed and irrigation treatments in Argentina, the grain weight simulation was in good agreement with the observation, with a root mean square of 0.0022 g (Savin et al., 1994). Two studies in Nebraska, US, reported that the normalized RMSEs for kernel weight simulations were about 0.003 g given planting population combined with sowing date treatments and about 0.0024 g given varied sowing date conditions (Moreno-Sotomayor and Weiss, 2004; Weiss and Moreno-Sotomayer, 2006). With five wheat production stations in the Czech Republic, the RMSE for a thousand-grain weight simulation was 7.5 g, which was within 20% error (Trnka et al., 2004). However, the thousand-grain weight was poorly simulated under combinations of CO₂ concentration and water availability treatments, with a model efficiency index of 0.44 (Biernath et al., 2011). Another study showed that the model underestimated the thousand-grain weight across experiments in humid and dry weather conditions in Algeria, with an average RMSE of 4.29 g for the model evaluation year (Rezzoug et al., 2008).

Table 8 Summary of the CERES-Wheat model performances for kernel weight^a and kernel number^a variable simulations.

Treatment category	Variables	Countries	Performance	References
Irrigated with a gradient of water/different scheduling time and well fertilized	Kernel weight	China Argentina	Percentage error: <9% Root mean square: 0.0022 g	He et al. (2013) Savin et al. (1994)
	Kernel number	Germany	Model efficiency index: 0.52	Biernath et al. (2011). ^b
Well irrigated, fertilized with a gradient of fertilizer(s) and combined with different sowing dates	Kernel number	Germany Netherlands	RMSE: 3677, R^2 : 0.07 RSME: 1808, R^2 : 0.77	Ratjen et al. (2012)
Well irrigated and well fertilized	Kernel weight	Argentina	RMSE: 0.0051 g R^2 : 0.32	Monzon et al. (2007)
		India	Percentage error: $\leq 13\%$	Hundal and PrabhjyotKaur (1997)
	Kernel number	Czech Republic	RMSE: 2845 Percentage error: <20%	Trnka et al. (2004)
Irrigated with a gradient of water and fertilized with a gradient of fertilizer(s) only	Kernel weight	Argentina Spain	RMSE: 3018 RMSE: 0.0076 g Mean percentage error: 22%	Monzon et al. (2007) Abeledo et al. (2008)
	Kernel number	Spain	RMSE: 4340 Mean percentage error: 24%	Abeledo et al. (2008)

Sowing dates	Kernel weight	United States	RMSE: 0.0023 ~ 0.0029 g Normalized RMSE: 9 ~ 12%	Weiss and Moreno-Sotomayer (2006) ^c ; Moreno-Sotomayer and Weiss (2004) Moreno-Sotomayer and Weiss (2004)
	Kernel number	United States	RMSE: 3998 ~ 4555 Normalized RMSE: 35 ~ 42%	

^aHere only showed individual wheat kernel weight and kernel number per square meter validation results; other relevant validated variables included thousand wheat kernel weight, and thousand kernel number, and the validation results were summarized in the text.

^bTreatment included two irrigation regimes (full irrigation vs. limit irrigation) combined with elevated CO₂ versus ambient CO₂ concentration.

^cTreatments include Planting density combined with sowing dates.

3.3.3 CERES-Rice

One study in Indonesia showed that the CERES-Rice model underestimated the kernel weight by almost 1 mg (Amien et al., 1999).

3.4 Kernel Number

3.4.1 CERES-Maize

Maize kernel number has been tested in the form of grain number per year, grain number per square meter, and grain number per plant. It has been validated with irrigation and fertilization treatments (Table 7). Soler et al. (2007) reported that the grain number simulation for both irrigated and rain-fed fields in Brazil were reasonably good, with average RMSEs of 102 and 257, respectively, and normalized RMSEs of 4.56 and 12.87%, respectively. Jagtap et al. (1993) showed that the CERES model could underestimate the number of grains per year by 21 or overestimate it by 68, depending on the year. With severe water stress, the simulated grain number per year was up to 21% less than the observations for 1 year but only about 1.7% less for another year (Ben Nouna et al., 2000; Mastrorilli et al., 2003). Across two water availability extremes, Retta et al. (1991) reported that the grain number per year simulation across dry- and irrigated-land treatments had an average RMSE of 94, and Carberry et al. (1989) calculated the RMSE to be 127.8 across well-watered and severe water stress treatments. In Argentina, for four sowing dates between late-Aug. and late-Nov., the CERES model overestimated the low-range grain numbers and underestimated the high-range grain numbers (Otegui et al., 1996). Six studies tested the grain number per square meter. Three of them suggested that in general, grain number on a square meter basis did not match very well with the observations under limited-water conditions, with a percentage difference up to 21% (Ben Nouna et al., 2000; Mastrorilli et al., 2003) and an RMSE over 1000 (Carberry et al., 1989). Another study tested grain number per square meter under rain-fed conditions in Nigeria. The grain number per square meter seemed to be reasonably well-simulated, with errors as low as 0 and as high as 174, depending on the simulation year (Jagtap et al., 1993). In Spain, kernel number per square meter simulations with cover crop treatments had a low error (RMSE < 590) as well (Salmerón et al., 2014). By contrast, the RMSE when simulating grain number per square meter under rain-fed conditions in Spain was over 1700 (López-Cedrón et al., 2008). In terms of grain number per plant tests, Lizaso et al. (2001) used 134 treatments over 4 years in Iowa and reported that the CERES consistently overestimated kernel number

when it was under 400 kernels per plant but underestimated kernel number per plant by 67 overall.

3.4.2 CERES-Wheat

Seven studies validated wheat kernel number under a range of treatments (Table 8). The reported average RMSE for grains per square meter ranged from 3998 to 4555 across irrigated and rain-fed fields in Spain (Abeledo et al., 2008) and for 31 sowing experiments in the United States (Moreno-Sotomayor and Weiss, 2004). By contrast, the RMSE ranged from 2845 to 3018 under fertilized condition in Argentina (Monzon et al., 2007) and the Czech Republic (Trnka et al., 2004). Under both water availability and CO₂ concentration treatments, the average model efficiency index for simulating grain number on a square-meter basis was 0.52 (Biernath et al., 2011). Ratjen et al. (2012) used two datasets from Germany with fertilization (0 ~ 320 kg N/ha) and sowing date treatments (Sep.–Oct.) and from the Netherlands under fertilization and sowing date treatments (varied sowing dates between Oct. 19 and 25) to test grain number per square meter and showed that the RMSEs were 3677 and 1808 grains/m², respectively. Savin et al. (1994) compared the standard error of the mean for the observed thousand-grain number to the root mean square of the simulated thousand-grain number across well-irrigated and early drought treatments, and the statistics were 1.34 and 0.56, respectively.

3.4.3 CERES-Rice

One simulation study used 32 experiments conducted under various locations, weather, and management conditions in India for over 13 years and showed that grain number per square meter was well-simulated when the number was over the range of 15,000–32,000 grains/m² (Mall and Aggarwal, 2002).

3.5 Aboveground Biomass and its Components (Excluding Grain Yield)

3.5.1 CERES-Maize

A total of 31 studies tested the biomass-related variables of the CERES-maize model, including aboveground biomass, LAI, stover biomass, biological yield, and shoot, leaf, stem, and year biomass (Tables 9 and 10). Under the maximum allowable depletion of available soil water in India, the average RMSE for aboveground biomass simulation was 202 kg/ha (Panda et al., 2004). In Turkey, with 50–100% sufficient water treatments, the model underestimated aboveground biomass by 2 ~ 8.6% (Gercek and Okant, 2010). The RMSEs

Table 9 Summary of the CERES-Maize model performances for aboveground biomass variable simulation.

Treatment category	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized	Spain	RMSE: >5000 kg/ha	López-Cedrón et al. (2008)
	Nigeria	RMSE: <400 kg/ha	Jagtap et al. (1993)
	Brazil	Normalized RMSE: 10 ~ 25%	Soler et al. (2007)
	China	Percentage error: 17%	Guo et al. (2010)
	Croatia	Percentage error: 3%	Vucetic (2011)
	United States	RMSE: 1701 kg/ha	Retta et al. (1991)
Irrigated with a gradient of water/different scheduling time and well fertilized	Turkey	Underestimated by 2 ~ 8.6%	Gercek and Okant (2010)
	Spain	Overestimated by 7%	Dechmi et al. (2010)
	India, Pakistan	RMSE: 202 ~ 1116kg/ha	Panda et al. (2004);
			Mubeen et al. (2013)
	United States, Spain	RMSE: 1600 ~ 1708 kg/ha	Saseendran et al. (2008),
			Dechmi et al. (2010)
	Australia	RMSE: over 5000 kg/ha	Carberry et al. (1989)
	United States	Normalized RMSE: 22 ~ 26%	Anothai et al. (2013)
	Italy	Percentage error: 14 ~ 30%	Ben Nouna et al. (2000), Mastrorilli et al. (2003)
Varied nitrogen levels, irrigated	China	Normalized RMSE: 15 ~ 23%	Liu et al. (2012)
Well irrigated and well fertilized	Spain	RMSE: 2202 kg/ha	López-Cedrón et al. (2005)
	Portugal	RMSE: 1494.17 kg/ha	Braga et al. (2008)
	United States, Brazil, Canada	Normalized RMSE: 23 ~ 33%	Xevi et al. (1996); Soler et al. (2007); Liu et al. (2014)
	Italy	Percentage error: 5.55%	Ben Nouna et al. (2000); Mastrorilli et al. (2003)

Not fertilized	Canada	Normalized RMSE: 41%	Liu et al. (2014)
Irrigated and fertilized at varied rates	United States	Percentage error: <12%	He et al. (2011)
	Iran	Normalized RMSE: 6.6%	Moradi et al. (2013)
Other treatments ^a	United States	Percentage error: ≤16%	Persson et al. (2009)
	United States	Percentage error: ≤17%	Saseendran et al. (2005)
Cover crops	Spain	RMSE: 530 ~ 3990 kg/ha	Salmerón et al. (2014)

^aOther treatments included sowing dates, planting density, and spacing.

Table 10 Summary of the CERES-Maize model performances for biomass-related variable (excluding aboveground biomass) simulations.

Treatment category	Variables	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized	Stover biomass	Nigeria	Differences: 3 ~ 701 kg/ha	Jagtap et al. (1993)
	Harvest index	Spain	Underestimated by 1.3	López-Cedrón et al. (2008)
		Croatia	Underestimated by 7%	Vucetic (2011)
Irrigated with a gradient of water/different scheduling time and well fertilized	Stover biomass	Australia	RMSD: 2421 kg/ha	Carberry et al. (1989)
	Harvest index	Spain	Underestimated by 13%	Dechmi et al. (2010)
Fertilized with different types of fertilizers	Shoot biomass	Thailand	Normalized RMSE: 16.8 ~ 79.1%	Pinitpaitoon et al. (2011)
Well irrigated and fertilized	Biological yield	Iran	Normalized RMSE: 4.22%	Moradi et al. (2014)
Irrigated with a gradient of water and fertilized with a gradient of fertilizer	Leaf biomass	United States	Agreement index: 0.65 ~ 0.85	Lizaso et al. (2011)
	Stem biomass		Agreement index: 0.85 ~ 0.9	
	Shoot biomass		Agreement index: >0.9	
	Year biomass		Agreement index: >0.93	
Planting densities	Biological yield	Iran	Normalized RMSE: 6.95%	Lashkari et al. (2011)

ranged from 1494.2 to 2202 kg/ha across 23 ~ 188 mm irrigation water treatments in Colorado, US (Saseendran et al., 2008), under well-irrigated and fertilized treatment in Spain (López-Cedrón et al., 2005) and under varied sowing dates treatments in Portugal (Braga et al., 2008). In contrast, the average RMSEs were over 5000 kg/ha across nonwater stress versus severe water stress treatments in Australia (Carberry et al., 1989) and rain-fed fields in Spain (López-Cedrón et al., 2008). When simulating biomass under seven irrigation treatments with different irrigation amounts and timings, the percentage errors were 6%, with average RMSEs over 1700 kg/ha (Mubeen et al., 2013). When simulating top biomass in seven maize production stations under rain-fed conditions in China, the percentage error was 17% (Guo et al., 2010). By comparison, for Croatia, the simulation error for rain-fed maize aboveground biomass was 3% (Vucetic, 2011). Under sowing date treatments (sown between late-Apr. and mid-Jun.), the percentage errors for biomass simulation were not larger than 17% (Saseendran et al., 2005). The simulated dry matter yield was within 12% absolute relative error given fertilization rates of 247 and 309 kg/ha and two irrigation (full irrigation and overirrigation) treatments, but the error was 29% given overirrigation with 185 kg N/ha addition treatment (He et al., 2011). Under low- and high-irrigation treatments in Spain, the total dry matter was overestimated by 7%, with an RMSE of 1650 kg/ha (Dechmi et al., 2010). Across 40 ~ 100%-full irrigation treatments in Colorado, US, the total biomass simulation had a normalized RMSE of at least 22.7% (Anothai et al., 2013). Under 141 ~ 219 kg N/ha fertilization rates with irrigation (irrigation and nonirrigation) and planting date (three dates across Mar.) treatments, the simulation errors were from 0.091 to 16.2% (Persson et al., 2009). Across three irrigation treatments (450 ~ 650 mm irrigation water) combined with four fertilization treatments (0 ~ 450 kg/ha) in Iran, the biomass yield simulation had a normalized RMSE of 6.6% and a model efficiency of 0.89 (Moradi et al., 2013). By comparison, under irrigated conditions in Nebraska, US, the normalized RMSE for simulating aboveground dry matter was 31.9% (Xevi et al., 1996). Others compared aboveground biomass simulation accuracy among treatments. The simulation research in Italy showed that with moderate and severe water stress, the CERES model consistently underestimated aboveground biomass by over 14% and over 24%, respectively, whereas it only underestimated the biomass by 5.55% without water stress (Ben Nouna et al., 2000; Mastrorilli et al., 2003). In Brazil, the aboveground biomass simulation for irrigated fields had normalized RMSEs between 23.6 and 32.9%, while that for rain-fed fields had normalized RMSEs between 10.1 and 24.7%

(Soler et al., 2007). A 2-year experiment in Kansas showed that the CERES model consistently underestimated aboveground vegetative biomass for irrigated fields but overestimated the biomass for dry-land fields. The average RMSE for the two fields was 1701 kg/ha, with a mean bias of 31 kg/ha (Retta et al., 1991). Under rain-fed conditions in Nigeria, the model overestimated the aboveground biomass by less than 400 kg/ha (Jagtap et al., 1993). Liu et al. (2012) reported that the normalized RMSEs were 23.1 and 15.4% when simulating maize aboveground biomass in the North China Plain given nitrogen application rates of 0 ~ 400 and 0 versus 180 kg N/ha, respectively. Using fertilized and unfertilized treatments in Canada, the normalized RMSEs for maize aboveground biomass were 26.8 and 41.2%, respectively (Liu et al., 2014). Given various winter cover-crop treatments, the aboveground biomass simulation under no cover crops and under winter cover crops of oilseed rape and vetch was in agreement with the observations, with an RMSE under 1600 kg/ha, but under winter rape and barley cover crop treatments, the simulations did not agree with the observations, with an RMSE over 2500 kg/ha (Salmerón et al., 2014).

Stover biomass, biological yield, shoot biomass, leaf biomass, stem biomass, shoot biomass, and year biomass have been validated as well. Under fully irrigated versus severe water stress treatments, the average RMSD when simulating maize stover biomass at maturity was 2421 kg/ha (Carberry et al., 1989). In rain-fed fields in Nigeria, the stover biomass was underestimated by 701 kg/ha for 1 year but overestimated by 3 kg/ha for another year (Jagtap et al., 1993).

In Iran, the normalized RMSE for biological yield was 6.95% under planting density treatments (Lashkari et al., 2011) and 4.22% under fertilized and irrigated treatments (Moradi et al., 2014). For shoot biomass, under mineral fertilizer and compost fertilizer treatments in Thailand, the model simulated maize shoot biomass relatively well for the second year but poorly for third ~ fifth years, with normalized RMSEs of 16.8% and 25.6 ~ 79.1%, respectively (Pinitpaitoon et al., 2011). Lizaso et al. (2011) tested leaf biomass, stem biomass, shoot biomass, and year biomass in Florida under rain-fed conditions with low nitrogen input (116 kg N/ha) and under irrigated conditions with high nitrogen input (401 kg N/ha), 0 and 240 kg N/ha, as well as with 56 and 224 kg N/ha application treatments in Iowa. The indices of agreement for the leaf biomass simulation were mostly above 0.85, with RMSEs from 584.8 to 1054.5 kg/ha, except for rain-fed fields with low nitrogen input and 0 kg N/ha application treatments in Florida (index of agreement below 0.65 and RMSEs of 1060.5 and 635.3 kg/ha, respectively).

By contrast, for stem biomass simulations, under rain-fed conditions with low nitrogen and 0 kg N/ha input treatments in Florida, the RMSEs were under 215 kg/ha, with index of agreement above 0.9, while under other treatments, the RMSEs were mostly above 1170 kg/ha, with agreement indexes around 0.85. The RMSEs for shoot biomass simulations ranged from 771.3 to 867.8 kg/ha for treatments in Florida and from 1647.7 to 2229.1 kg/ha in Iowa. The year biomass was simulated only for 0 kg N/ha and 240 kg N/ha treatments in Florida and nitrogen application treatments in Iowa. The indices of agreement were all above 0.93, with RMSEs of 396.5 ~ 1155.4 kg/ha (Lizaso et al., 2011).

3.5.2 CERES-Wheat

The aboveground biomass prediction capability of the CERES-Wheat model has been evaluated under several treatments, including water availability treatments, fertilization treatments, sowing date treatments, CO₂ concentration treatments, and management intensity treatments (Table 11). Under rain-fed conditions in India, the aboveground biomass simulation had an index of agreement of 0.98 and an r^2 of 0.95 (Vashisht et al., 2013). The average RMSEs were 333 and 900 kg/ha across irrigation treatments at 30 ~ 75% of the maximum allowable soil water depletion in India (Panda et al., 2003) and across irrigation with various amounts water and at various developmental stages in New Zealand (Jamieson et al., 1998). On average, the CERES-Wheat model underestimated the aboveground biomass by 644 kg/ha for seven production stations in China (Guo et al., 2010). Given different irrigation applications (from no irrigation to full irrigation) combined with different nitrogen fertilizer application rates (from zero to a high rate), the reported RMSEs ranged from 1200 to 2360 kg/ha (Abeledo et al., 2008; Arora et al., 2007; Singh et al., 2008). Under rain-fed and fertilization treatments (0 ~ 112 kg N/ha) in the United States, the average RMSE was 1247 kg/ha. Under well-fertilized conditions with conventional and no-till management, the RMSEs were 1999 and 2282 kg/ha, respectively (Saseendran et al., 2004; Soldevilla-Martinez et al., 2013). The average normalized RMSE was 9.5% across two nitrogen application treatments (110.5 and 241 kg N/ha) in the United States (Thorpe et al., 2010b), and it was within 19% when simulating aboveground biomass under a CO₂ concentration treatment combined with irrigation treatment (Tubiello et al., 1999b) and 23.1% across three rotation experiments in India, including a wheat-rice field under nitrogen stress treatment (0 ~ 160 kg N/ha) and another wheat-rice field, a wheat-maize, and wheat-soybean fields with sufficient fertilizer input

Table 11 Summary of the CERES-Wheat model performances for aboveground biomass variable simulations.

Treatment category	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized	India	R^2 : 0.95	Vashisht et al. (2013)
Irrigated with a gradient of water/different scheduling time and well fertilized	India	RMSE: 333 kg/ha	Panda et al. (2003)
	New Zealand	RMSE: 900 kg/ha	Jamieson et al. (1998)
Well irrigated and fertilized with a gradient of fertilizer(s)	United States	RMSE: 1247 kg/ha Normalized RMSE: 9.5%	Saseendran et al. (2004) Thorp et al. (2010b)
	India	Percentage error: 23%	Timsina et al. (2008)
Well irrigated and not fertilized	Germany	R: 0.8	Bacsi and Zemankovics (1995)
Well irrigated and well fertilized ^a	China	Underestimated by 644 kg/ha Percentage error: 11.4%	Guo et al. (2010)
Irrigated with a gradient of water and fertilized with a gradient of fertilizer(s)	India	RMSE: 1200 ~ 1940 kg/ha Normalized RMSE: 14%	Singh et al. (2008); Arora et al. (2007)
	Spain	RMSE: 2360 kg/ha Percentage error: 17%	Abeledo et al. (2008)
Conventional versus no tillage	United States	1999 ~ 2282 kg/ha	Soldevilla-Martinez et al. (2013)
CO ₂ concentration with irrigation regimes	United States	Percentage error: 5 ~ 19%	Tubiello et al. (1999b)
CO ₂ concentration	Germany	Normalized RMSE: 27%	Biernath et al. (2011)
	United States	Overestimated by 20 kg/ha	Tubiello et al. (1999a)

Sowing date	India	Percentage error: <20% (for over 80% of the simulations)	Hundal and PrabhjyotKaur (1997)
	Germany	Percentage error: <8%, $R^2 > 0.95$	Bacsi and Zemankovics (1995)
Planting density combined irrigation treatments; phosphorous input with seeding rates	Iran	Normalized RMSE: 4.8%	Bannayan et al. (2014)
Wheat with different fallow scheduling systems	Canada	R: 0.85	Wang et al. (2010)

^aLiteratures that did not include treatments were considered as “well irrigated and well fertilized”; for instance, data obtained from local reports.

(Timsina et al., 2008). With continuous wheat and rotation wheat cropping systems in Canada, the correlation coefficients between the simulated and the observed biomass were above 0.8 (Wang et al., 2010). Hundal and PrabhjotKaur (1997) reported that the CERES model tended to overestimate aboveground biomass for both early- and late-sowing date treatments, but over 80% of the simulated aboveground biomass was within 20% error. Across four experiments with three sowing dates (DOY273, DOY284 and DOY289) and an unfertilized treatment with a sowing date of DOY289, the CERES model also overpredicted the total aboveground biomass at harvest, and the errors were mostly within 8%, except for the fertilized DOY289 treatment (Bacsi and Zemankovics, 1995). Across treatments with different planting densities, irrigation inputs, phosphorous levels, and seeding rates in Iran, the normalized RMSE for aboveground biomass was 4.8% (Bannayan et al., 2014). Under an elevated CO₂ concentration, the simulated aboveground biomass was overestimated by 20 kg/ha (Tubiello et al., 1999a). Across elevated and ambient CO₂ concentration treatments combined with full versus limited irrigation treatments, the average normalized RMSE was 27% for total aboveground biomass simulation (Biernath et al., 2011).

Other biomass-related variable validations, including vegetative biomass, organ biomass, straw biomass, canopy biomass, total biomass, leaf biomass, biological yield, harvest biomass residual, harvest biomass, and surface residue biomass have been reported in the literature (Table 12). Priesack et al. (2006) tested the CERES-Wheat model at an extensive managed plot to which a mixture of inorganic and organic fertilizer was applied and on which a low level of chemical plant protection was used. They reported that the simulated vegetative aboveground and organ storage biomass were close to the observations. Straw biomass has been tested with various fertilization rates. Under nutrient stress (different ratios of nitrogen, phosphorus, and potassium) combined with water stress treatments, the wheat straw biomass simulation had RMSEs of 227.9 and 318.6 kg/ha, depending on the year (Behera and Panda, 2009). Across two types of rotation cropping systems with nutrient stress, the average normalized RMSD was 39.9% (Timsina et al., 2008). Under irrigated and fertilized fields in Arizona, US, the canopy biomass simulation had a normalized RMSE of 18.6% (Thorp et al., 2012). For six experiments involving nitrogen application (about 80 and about 215 kg/ha) and planting density treatments in Arizona, the errors varied greatly, by up to 2000 kg/ha (Thorp et al., 2010a). Across two nitrogen fertilizer treatments in Arizona, the normalized RMSE for leaf mass at the end of the leaf development simulation was 12% (Thorp et al., 2010b). Bakhsh et al. (2013) reported that

Table 12 Summary of the CERES-Wheat model performances for biomass-related variable (excluding aboveground biomass) simulations.

Treatment category	Variables	Countries	Performance	References
Well irrigated and fertilized with a gradient of fertilizer (s)/different types of fertilizers	Straw biomass	India	Normalized RMSE: 40%	Timsina et al. (2008)
	Harvest index	India	RMSE: 0.135	Timsina et al. (2008)
			Normalized RMSE: 35.1%	
	Leaf biomass	United States	Normalized RMSE: 12%	Thorp et al. (2010)b
Well irrigated and well fertilized ^a	Biological biomass	Pakistan	RMSE: <770 kg/ha	Bakhsh et al. (2013)
	Canopy biomass	United States	Normalized RMSE: 18.6%	Thorp et al. (2012)
Irrigated with a gradient of water and fertilized with a gradient of fertilizer(s)	Total biomass	Spain	RMSE: 2360 kg/ha Mean percentage error: 17%	Abeledo et al. (2008)
	Straw biomass	India	RMSE: 227 ~ 318 kg/ha; R^2 : 0.96 ~ 0.97	Behera and Panda (2009)
	Harvest index	Spain	RMSE: 0.16; percentage error: 24%	Abeledo et al. (2008)
CO ₂ concentration	Harvest index	United States	Percentage error: 6%	Tubiello et al. (1999a)
Planting density combined with high versus low nitrogen	Canopy biomass	United States	Difference: <2000 kg/ha	Thorp et al. (2010a)
Rotation systems	Harvest biomass	Germany	RMSE: 2100 kg/ha	Langensiepen et al. (2008)
	Harvest biomass residuals		RMSE: 2200 ~ 2400 kg/ha	

(Continued)

Table 12 Summary of the CERES-Wheat model performances for biomass-related variable (excluding aboveground biomass) simulations.—
cont'd.

Treatment category	Variables	Countries	Performance	References
Management insensitivity	Vegetative biomass and organ storage biomass	Germany	The simulations were close to the observations	Priesack et al. (2006)
Wheat with different fallow scheduling systems	Surface residue biomass	Canada	Error: 1800 kg/ha underestimation ~ 2400 kg/ha overestimation	Wang et al. (2010)

^aLiteratures that did not include treatments were considered as “well irrigated and well fertilized”; for instance, data obtained from local reports.

the RMSEs for biological yield simulations of 11 fertilization treatments with different amounts and types of fertilizers in Pakistan were within 770 kg/ha. Predictions for harvest biomass residual and harvest biomass were validated using 10-year data from a wheat-barley-canola rotation field in Germany. The RMSEs for harvest biomass residual ranged between 2200 kg/ha and 2400 kg/ha, depending on the genotype calibration processes, while the RMSEs for harvest biomass were about 2100 kg/ha in spite of the different calibration processes (Langensiepen et al., 2008). In continuous and rotation wheat systems in Canada, the surface residue biomass simulation had errors ranging from 1800 kg/ha underestimation to 2400 kg/ha overestimation (Wang et al., 2010).

3.5.3 CERES-Rice

Five studies validated aboveground biomass and the top weight of the CERES-Rice model (Table 13). Under both irrigation treatments (five irrigation levels ranging from 625 to 1225 mm) and seedling density treatments (3 density levels of 1 ~ 3 seedling/hill), the average RMSE was 385 kg/ha (Ahmad et al., 2012). Two studies in Thailand validated aboveground biomass/top weight under nitrogen application treatment. The authors reported that for each nitrogen application treatment (0 ~ 150 kg/ha), the absolute percentage errors were within 25% and the simulations were more accurate for 50 ~ 100 kg N/ha treatments (errors were within 10%) (Cheyglinted et al., 2001). Also, the average RMSE for top weight simulation under 0 ~ 188 kg N/ha application treatments was 1103 kg/ha, with agreement index of 0.98 (Phakamas et al., 2013). Assembling 32 experiments with 80 treatments, including planting dates, planting populations, spacings, nitrogen application rates, and irrigation rates, Mall and Aggarwal (2002) found that overall, the CERES model slightly underestimated the aboveground biomass, especially in the early crop growing stage (Mall and Aggarwal, 2002).

Additionally, by-product biomass prediction with the CERES-rice model was tested with late-seeded rice in Thailand, with a simulation error of 3.13% (Babel et al., 2011). Shoot biomass was validated in an open-field and elevated-CO₂ environment, and the normalized RMSEs were between 8 and 19% (Satapathy et al., 2014).

3.6 Harvest Index

3.6.1 CERES-Maize

For fertilized and rain-fed fields in Spain and Croatia, the harvest index was underestimated by 1.3, with an RMSE of 0.155 (López-Cedrón et al., 2008),

Table 13 Summary of the CERES-Rice model performances for biomass-related variable simulations.

Treatment category	Variables	Countries	Performance	References
Well irrigated and fertilized with a gradient of fertilizer(s) only	Aboveground biomass	Thailand	Percentage error: <25%	Cheyglinted et al. (2001) Phakamas et al. (2013)
	Top weight	Thailand	RMSE: 1103 kg/ha R^2 : 0.98	
Fertilized, irrigated with varied amount of water and planted with varied densities	Aboveground biomass	India	RMSE: 137 ~ 174 kg/ha	Ahmad et al. (2012) Ahmad et al. (2012)
	Top weight	India	RMSE: 385 kg/ha	
Over 80 treatments ^a	Aboveground biomass	India	Close to the observations except for early growth stage (up to panicle initiation)	Mall and Aggarwal (2002)
Late-seeded rice	Byproduct biomass	Thailand	Error: 3.13%	Babel et al. (2011)
	Harvest index		Error: 5%	
Open filed and elevated CO ₂	Shoot biomass	India	Normalized RMSE: 8 ~ 19%	Satapathy et al. (2014)

^aTreatments included varied seeding and transplanting dates, planting densities, spacing, nitrogen inputs, and irrigations.

and by 7% (Vucetic, 2011), respectively. Across irrigation treatments of 421 ~ 609 mm in Spain, the harvest index was underestimated by 13%, with an RMSE of 0.08 (Dechmi et al., 2010) (Table 10).

3.6.2 CERES-Wheat

Three studies tested the harvest index (Table 11). Across all irrigated versus rain-fed and fertilized versus nonfertilized treatments, the overall RMSE was 0.16, and the mean percent error was 34% (Abeledo et al., 2008). Under various rotation cropping systems and different types of soils, with one of the cropping systems using an unfertilized treatment, the HI simulation had an absolute RMSE of 0.135 and a normalized RMSE of 35.1% (Timsina et al., 2008). In contrast, the error was 6% in an elevated CO₂ environment (Tubiello et al., 1999a).

3.6.3 CERES-Rice

The simulated harvest index was only about 5% off of the observation for a late-sown rice field in Thailand (Babel et al., 2011).

3.7 Leaf-Related Variables: Leaf Number, Leaf Senescence, and LAI

3.7.1 CERES-Maize

The leaf number and LAI prediction capabilities of the CERES-Maize model have been validated under irrigation, fertilization, sowing date, planting density, and spacing treatments in various countries (Tables 14 and 15). Carberry et al. (1989) tested leaf number and leaf senescence under irrigated versus severe water stress treatments in Australia. The authors found that the overall RMSE for leaf number simulation was 2.49 and that the model did not simulate leaf area senescence accurately during the growing season (Carberry et al., 1989). Gungula et al. (2003) simulated leaf number at anthesis date given 0 ~ 120 kg N/ha treatments and indicated that the simulated leaf numbers were close to the observations given 60 ~ 120 kg N/ha application treatments. Braga et al. (2008) reported that the RMSE for total leaf number simulation was 0.87.

Twenty-three papers have validated the LAI of the CERES-Maize model (Table 13). Under nonirrigated treatment, the simulated LAI was close to the observed LAI during the growing season (Sandor and Fodor, 2012). With various amounts of irrigation water and various irrigation-timing treatments, the model simulated LAI reasonably well throughout the growing season, with an agreement index over 0.8, and the simulated maximum LAIs

Table 14 Summary of the CERES-Maize model performances for LAI variable simulations.

Treatment category	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized	Brazil	Percentage error: 10 ~ 24%	Soler et al. (2007)
	Nigeria, India, Croatia	Percentage error: $\leq 6\%$	Jagtap et al. (1993) ; Panda et al. (2004) ; Vucetic (2011)
	Hungary	Close to the observations	Sandor and Fodor (2012)
Irrigated with a gradient of water/ different scheduling time and well fertilized	China	Error: 0.5 ~ 0.7	Guo et al. (2010)
	Italy	Percentage error: 26 ~ 46%	Ben Nouna et al. (2000) ; Mastrorilli et al. (2003)
	Pakistan	Percentage error: 5.9 ~ 23%	Mubeen et al. (2013)
	United States, India	Percentage error: $< 5\%$	DeJonge et al. (2011) ; Panda et al. (2004)
	India	RMSE: < 0.2	Panda et al. (2004)
	Pakistan, United States	RMSE: 0.68 ~ 0.88	Mubeen et al. (2013) ; DeJonge et al. (2011)
	Australia, United States	RMSE: 0.9 ~ 1.14	Carberry et al. (1989) ; Retta et al. (1991)
	Spain	Closed to the observed in the early growing stages but not late growing stages	Dechmi et al. (2010)
Well irrigated and well fertilized	United States	RMSE: 0.307	DeJonge et al. (2011)
	Italy	Percentage error: 0.97%	Ben Nouna et al. (2000) ; Mastrorilli et al. (2003)
	Brazil, China	Percentage error: 10% ~ 24%	Soler et al. (2007) ; Guo et al. (2010)
	United States	Normalized RMSE: 35.7%	Xevi et al. (1996)
	Spain	RMSE: 1.2 ~ 2	López-Cedrón et al. (2005)
	Canada	Normalized RMSE: 14 ~ 50%	Liu et al. (2014)

Not fertilized Irrigated with a gradient of water and fertilized with a gradient of fertilizer(s) Other treatments ^a	Canada	Normalized RMSE: 65 ~ 98%	Liu et al. (2014)
	Iran	Normalized RMSE: 5.22%	Moradi et al. (2013)
	United States	Poorly simulated	Lizaso et al. (2001); Lizaso et al. (2003b)
	United States	RMSE: 0.33 ~ 1.47	Saseendran et al. (2005); Lizaso et al. (2011); Lizaso et al. (2003a)
	Iran	RMSE: 12.79 R^2 : 0.94	Lashkari et al. (2011)

^aOther treatments included sowing dates, planting density, and spacing.

Table 15 Summary of the CERES-Maize model performances for leaf number variable simulations.

Treatment category	Variables	Validation Nations	Performance	References
Irrigated with a gradient of water/different scheduling time and well fertilized	Leaf number	Australia	RMSE: 2.49	Carberry et al. (1989)
Well irrigated and fertilized with a gradient of fertilizer(s)	Leaf number at anthesis	Nigeria	Difference: under-predicted by 0 ~ 5 Percentage error: 0 ~ 17%	Gungula et al. (2003)
Sowing dates	Total leaf number	Portugal	RMSE: 0.87	Braga et al. (2008)

were within 23% error (Mubeen et al., 2013). Similarly, for both full and limited irrigation treatments, DeJonge et al. (2011) reported that the LAIs were overestimated over the growing season but were underestimated during the reproductive stage. The authors also compared the model accuracy for final LAI simulation between treatments and showed that the model simulated the LAI better for full irrigation treatments (RMSE = 0.307) than limited irrigation treatments (RMSE = 0.841) (DeJonge et al., 2011). Up to 26.96 and 46.15% underestimation of maximum LAI were found under moderate and severe water stress, respectively, but only a 0.97% overestimation was found under full irrigation condition (Ben Nouna et al., 2000; Mastrorilli et al., 2003). By comparison, when simulating LAI in rain-fed and irrigated fields in Brazil with four different maize cultivars, the normalized RMSEs were 10.4 ~ 24.2% and 10.9 ~ 24.4%, respectively (Soler et al., 2007). Others reported that the average difference between simulated and observed LAI was no greater than 0.09 under rain-fed and fertilized condition (Jagtap et al., 1993) and various levels of water availability (Panda et al., 2004). In contrast, the difference between the simulations and the observations was about 0.2 for a rain-fed, fertilized treatment in Nigeria (Jagtap et al., 1993) and an irrigated treatment in the United States (Xevi et al., 1996). Across rain-fed fields in Croatia, the model underestimated the maximum LAI by 4% (Vucetic, 2011). LAI was poorly simulated for both fertilized and unfertilized maize in Canada, with normalized RMSEs of 14 ~ 50% and 65 ~ 98%, respectively (Liu et al., 2014). Guo et al. (2010) indicated that the maximum LAI was underestimated by about 0.7 and that the mean LAI was underestimated by about 0.5 for seven stations in China. LAI during the silking developmental stages has been mostly poorly simulated under various treatments in Iowa, including planting dates, nitrogen application rates, fertilization rates, and population densities (Lizaso et al., 2001, 2003b). The LAI at silking simulation had an RMSE of 1.14 across severe water stress and full irrigation treatments in Australia (Carberry et al., 1989). The RMSEs for the LAI simulations were 0.33 and 0.84 for full irrigation and limited irrigation, respectively (DeJonge et al., 2011). The average RMSE for LAI simulation in both dry- and irrigated-land in Kansas, US, was 0.9 (Retta et al., 1991). However, with the maximum allowable depletion of available soil water, 30 ~ 75%, the average RMSE for LAI simulations was 0.194 (Panda et al., 2004). Dechmi et al. (2010) showed that early growing season LAIs were well-simulated for low- and high-irrigation treatments but that the maximum LAIs were underpredicted. The reported average RMSE for

three maize hybrid LAI simulations ranged from 0.33 to 0.78 for three sowing dates between the end of Apr. and mid-Jun. in Colorado, US (Saseendran et al., 2005). The reported average normalized RMSE for the maximum LAI simulation of an irrigation treatment combined with a fertilization treatment in Iran was 5.22% (Moradi et al., 2013). However, others showed that the simulations of LAI were less accurate. For a population density treatment in Minnesota, nitrogen application in Hawaii, and in rain-fed and irrigated fields in Florida, LAI was not well-simulated, with RMSEs from 0.33 to 1.47 (Lizaso et al., 2003a, 2011). The RMSEs for LAI simulations in an experimental site in Spain were 1.21, as best result (López-Cedrón et al., 2005). Lashkari et al (2011) calculated the average RMSE for maximum LAI simulation under planting density treatments, and the value was 12.79 (Lashkari et al., 2011). Xevi et al. (1996) reported a normalized RMSE of 31.9% for LAI simulation for irrigated maize in Nebraska.

3.7.2 CERES-Wheat

One study tested leaf number with nitrogen application treatments in Arizona, US, and showed that leaf number development was reasonably well-simulated (Thorp et al., 2010b).

Fifteen studies reported LAI validation results for the CERES-Wheat model (Table 16). Bacsí et al. (1995) showed that LAI was simulated reasonably well during the course of development given a nonfertilized treatment (Bacsí and Zemankovics, 1995). However, a study in Arizona, US, with various levels of nitrogen input and planting density indicated that the predicted green LAI did not match well with the observations (Thorp et al., 2010a). The average difference between simulated and observed LAI ranged between 0.016 and 0.12 under various conditions, including various combinations of water availability and N:P:K ratios (Behera and Panda, 2009), seven wheat and maize production sites in China (Guo et al., 2010), and various levels of water availability (Panda et al., 2003). However, given 0 ~ 4 irrigation treatments in China, the differences between the simulated and the observed LAI were between 0.3 and 0.6 (Yang et al., 2006b). Given various combinations of CO₂ concentration and irrigation level, the normalized RMSE for simulated LAI was 1.27% (Biernath et al., 2011). Across different planting densities, irrigation inputs, phosphorous levels, and seeding rates in Iran, the normalized RMSE for LAI was 8% (Bannayan et al., 2014). By contrast, across various levels of irrigation combined with different fertilization application rates, the average RMSEs for LAI at 32, 54, 82, and 124 days after planting were 0.1, 0.5, 0.9, and 0.6, respectively (normalized RMSE of 25 ~ 35%) (Arora et al., 2007).

Table 16 Summary of the CERES-Wheat model performances for LAI simulations.

Treatment Category	Countries	Performance	References
Irrigated with a gradient of water/different scheduling time and well fertilized	India	RMSE: 0.108 Percentage error: 1.14% R^2 : 0.92	Panda et al. (2003)
	New Zealand	The simulated LAI did not respond to drought factor	Jamieson et al. (1998)
	China	Error: 0.3 ~ 0.6	Yang et al. (2006b)
Well irrigated and fertilized with a gradient of fertilizer(s) only	United States	Normalized RMSE: 17.9%	Thorp et al. (2010b)
Well irrigated and well fertilized	China	Underestimated mean LAI by 0.5	Guo et al. (2010)
	United States	Normalized RMSE: 27.8%	Thorp et al. (2012)
Irrigated with a gradient of water and fertilized with a gradient of fertilizer(s)	India	RMSE: 0.069 ~ 0.075 R^2 : > 0.9	Behera and Panda (2009)
	India	RMSE: 0.1 ~ 0.9 Normalized RMSE: 25 ~ 35%	Arora et al. (2007) ^a
	China	RMSE: 0.87 (for all LAIs), 0.67 (for LAI \geq 3) Normalized RMSE: 20%	Dong et al. (2013)a; Dong et al. (2013)b; Ji et al. (2014)
	Iran	Normalized RMSE: 8%	Bannayan et al. (2014)
Planting density combined irrigation treatments; phosphorous input with seeding rates	United States	Not accurate	Thorp et al. (2010a)
Planting densities combined with high versus low nitrogen	Germany	Normalized RMSE: 1.27%	Biernath et al. (2011)
CO ₂ concentration combined with two irrigation treatments	Germany	R^2 : 0.571	Bacsi and Zemankovics (1995)
Sowing date combined with different nitrogen input			

^aTreatments included different irrigation regimes combined with fertilization regimes and four planting dates.

Ji et al. also reported that the normalized RMSE for LAI simulation was about 20% under varied nutrient and water input treatments in China (Ji et al., 2014). Similarly, the normalized RMSEs were 17.9 and 27.8% for LAI simulations under nitrogen application treatments (110.5 kg/ha vs. 241 kg N/ha) and no nutrient stress treatments, respectively, in Arizona, US (Thorp et al., 2010b; 2012). For water availability treatments, including full irrigation, early drought, late drought, and full drought, the CERES model underestimated LAI for most of the treatments, and the simulated LAI did not respond to the drought factor (Jamieson et al., 1998). Additionally, Dong et al. (2013b) found that the CERES-Wheat model overestimated LAI, particularly when the LAIs were less than 3. With four irrigation treatments ($0 \sim 675 \text{ m}^3/\text{ha}$) and four fertilization treatments ($0 \sim 225 \text{ kg N/ha}$), Dong et al. (2013a) also reported that the RMSEs for all LAIs and $\text{LAI} \geq 3.0$ simulations were 0.87 and 0.67, respectively.

3.7.3 CERES-Rice

Only two studies tested the LAI variable in the CERES-Rice model (Table 17). Mall and Aggarwal (2002) used data from 32 experiments, which consisted of planting date, planting density, spacing, irrigation, and nitrogen application treatments, and showed that overall, the model simulated LAI well but slightly underestimated LAI, particularly around the flowering stage. Under irrigation and planting density treatments, the RMSEs for LAI simulation were mostly under 1.3 and were 1.12 on average (Ahmad et al., 2012).

3.8 Soil Nitrogen

Soil nitrogen content and nitrate leaching prediction have been validated for the CERES-Maize and CERES-Wheat models. No research has

Table 17 Summary of the CERES-Rice model performances for LAI simulations.

Treatment category	Countries	Performance	References
Over 80 treatments ^a	India	Overall accurate but underestimated LAI around flowering stage	Mall and Aggarwal (2002)
Fertilized, irrigated with varied amount of water and planted with varied densities	India	RMSE: 1.08 ~ 1.33, Average RMSE: 1.12	Ahmad et al. (2012)

^aTreatments included varied seeding and transplanting dates, planting densities, spacing, nitrogen inputs, and irrigations.

reported on soil nitrogen prediction validation for the CERES-Rice model.

3.8.1 CERES-Maize

Soil nitrogen content and nitrate leaching have been tested in both continuous cropping systems and rotation systems using nitrogen availability, legume cover crop incorporation, and irrigation treatments (Table 18). Gabrielle and Kengni (1996) simulated soil mineral nitrogen content using the CERES model for five experiments at three sites over 2 years in France: a Grenoble site with two irrigated fields, one with and one without fertilization; a Laon site with one tilled field with canola straw removal and another with the straw remaining; and a Grignon site with no carbon or nitrogen input. The results showed that the simulated nitrogen for 0–90 or 0–120 cm soil did not match the measured results and mostly underestimated soil nitrogen content. The RMSE of nitrogen content in the 0–30 cm soil was up to 159 kg/ha for the nonfertilized Grenoble experiment and as low as 8.5 kg/ha for the Grignon experiment. The authors also simulated nitrate leaching for the Grenoble site. They reported that the RMSEs were 21.3 and 8.4 kg/ha for the unfertilized and fertilized experiments, respectively (Gabrielle and Kengni, 1996). Given three levels of nitrogen input for 2 years (20 ~ 280 kg/ha for 1 year and 30 ~ 270 kg/ha for the other year), the simulated nitrate leaching was significantly different from the observed leaching ($P \leq 0.05$) (Pang et al., 1998). A similar study with 0 ~ 200 kg N/ha input treatments in tropical Thailand indicated that the model tended to underestimate nitrate leaching, with a coefficient of determination of 0.86 (Asadi and Clemente, 2003). Another study in Canada showed that the model performed better for soil inorganic nitrogen simulations given afertilized maize treatments (normalized RMSE: 35.8 ~ 57.1%) than unfertilized maize treatments (normalized RMSE: 72 ~ 81%) (Liu et al., 2014). Furthermore, by simulating soil mineral nitrogen content for a year and nitrate–nitrogen loss for 3 years in both fertilized and unfertilized plots in Canada, Liu et al. (2010) calculated that the RMSEs for simulating soil nitrogen content at 0 ~ 13 cm were 2 and 1.3 kg/ha for the fertilized and the unfertilized plots, respectively, with normalized RMSEs of 58 and 64%, respectively. They also observed a consistent overestimation of soil nitrate leaching through subsurface tiles for unfertilized plots, with 160% of normalized RMSE. By comparison, in fertilized plots, the nitrogen loss was reasonably well-simulated, with a normalized RMSE of 29% and an RMSE of 12.8 kg/ha (Liu et al., 2010). Nonetheless, nitrate leaching was well-simulated for unfertilized plots and no-till plots in a study

Table 18 Summary of the CERES-Maize model performances for soil nitrogen and nitrate leaching simulations.

Treatment category	Variables	Countries	Performance	References
Well irrigated and fertilized with a gradient of fertilizer(s)	Nitrate leaching	United States	R^2 : 0.5, significantly different from the observed ($P \leq 0.05$)	Pang et al. (1998)
		Canada	Matched well with the observations	Beckie et al. (1995)
		Thailand	R^2 : 0.86	Asadi and Clemente (2003)
Well irrigated and not fertilized	Soil nitrogen content	Hungary	Close to the observed	Kovacs et al. (1995)
		Hungary	Close to the observed	Kovacs et al. (1995)
	Soil nitrogen content, 0–30cm	France	RMSE: 159 kg/ha	Gabrielle and Kengni (1996)
		Canada	RMSE: 1.3 kg/ha	Liu et al. (2010),
	Soil nitrogen content, 0–13cm and 0–30 cm		Normalized RMSE: 64 ~ 81%	Liu et al. (2014)
		France	RMSE: 21.3 kg/ha	Gabrielle and Kengni (1996)
Well irrigated and well fertilized	Nitrate leaching	Canada	RMSE: 8.2 kg/ha	Liu et al. (2010)
		Canada	Normalized RMSE: 160%	Liu et al. (2010)
		France	RMSE: 8.5 kg/ha	Gabrielle and Kengni (1996)
	Soil nitrogen content, 0–30 cm			
		Canada	RMSE: 2 kg/ha	Liu et al. (2010),
	Soil nitrogen content, 0–13 cm and 0–30 cm		Normalized RMSE: 30 ~ 34%	Liu et al. (2014)
		Canada	Normalized RMSE: 58%	Liu et al. (2010)
	Nitrate leaching	France, Canada	RMSE: 8.4 ~ 12.8 kg/ha	Gabrielle and Kengni (1996); Liu et al. (2010)
		Canada	Normalized RMSE: 29%	Liu et al. (2010)

Irrigated with a gradient of water and fertilized with a gradient of fertilizer(s)	Nitrate leaching	United States	Difference: 10 ~ 40 kg/ha	He et al. (2011)
Corn-alfalfa-corn rotation with and without fertilization	Nitrate leaching	United States	Unfertilized corn field nitrate leaching was well simulated; did not well simulate alfalfa effect on nitrate leaching; did not simulate tillage effect either	Gerakis et al. (2006)
Wheat-maize rotation with legume cover crop	Soil nitrogen	United States	Underestimated by 25 ~ 150 kg/ha	Hasegawa et al. (2000)
Soil types	Soil nitrate content	United States	RMSE: <8 $\mu\text{NO}_3^-/\text{soils}$	Garrison et al. (1999)

of a corn-alfalfa-corn rotation field in the Midwest of the United States. However, the model failed to simulate nitrate leaching in tilled fields and after alfalfa growth (Gerakis et al., 2006). Beckie et al. (1995) mentioned that the simulated nitrate leaching matched well with the observations for two wheat fields with and without fertilization in Canada (Beckie et al., 1995). Kovacs et al. (1995) indicated that the largest errors in nitrate leaching simulation occurred with unfertilized treatments. In their study, maize–wheat rotation fields in Hungary were fertilized with 0, 50, 150, or 250 kg/ha of nitrogen, in addition to phosphorus and potassium addition, and nitrate leaching was measured in 4 ~ 5 m soils. The test results showed that over 20 years, soil nitrogen balance and accumulative nitrate leaching simulations were in good agreement with field measurements (Kovacs et al., 1995). Garrison et al. (1999) calculated RMSEs for soil nitrate content under fertilized maize fields with two different soils and reported that the RMSEs were within 8 $\mu\text{g-NO}_3^-/\text{soils}$. For soil nitrogen simulation for a wheat–maize rotation with LCC incorporation cropping systems in the United States, the CERES–Maize model underestimated soil nitrogen by 25–150 kg N/ha in 1 year and by 25–55 kg N/ha in another year under early LCC incorporation conditions (Hasegawa et al., 2000). For six treatments combining three levels of nitrogen input (185 ~ 309 kg/ha) and two levels of irrigation water input (irrigating water use depending on soil moisture versus 1.5 times the first irrigation water use), the CERES model underestimated potential nitrate leaching for the low-nitrogen input treatment, with 10 and 31 kg/ha error for normal and overirrigated treatment, respectively, and it overestimated potential nitrate leaching for the higher nitrogen input treatment, with about 40 and about 10 kg/ha error for the normal- and over-irrigated treatments, respectively (He et al., 2011).

3.8.2 CERES-Wheat

Five studies validated the soil nitrogen variables of the CERES–Wheat model under varied treatments and rotation systems (Table 19). Popova et al. (2005) tested soil nitrate–nitrogen in two soil types with 200 kg N/ha input combined with a range of irrigation water input, from 0 to 183 mm, and reported that the coefficients of determination were 0.38 and about 0.45 for a maize field soil nitrate simulation given nonirrigated and irrigated treatments, respectively (Popova and Kercheva, 2005). When simulating soil nitrogen in a wheat–maize rotation with LCC incorporation experiment, Hasegawa et al. (2000) found that the simulated inorganic nitrogen content in the soil was within 20% error for unfertilized fallow–wheat and wheat–legume rotation systems. Beckie et al. (1995) also indicated that the total and

Table 19 Summary of the CERES-Wheat model performances for soil nitrogen and plant nitrogen simulations.

Treatment category	Variables	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized	Soil nitrate-N	Bulgaria	Reasonably well simulated	Popova and Kercheva, (2005)
Well irrigated and fertilized with a gradient of fertilizer(s)	Plant nitrogen content	United States	Normalized RMSE: 10.9%	Thorp et al. (2010b)
Varied levels of irrigation and nitrogen	Canopy nitrogen	China	Normalized RMSE: 20%	Ji et al. (2014)
Well irrigated and well fertilized only ¹	Plant nitrogen content	United States	Normalized RMSE: 50.7%	Thorp et al. (2012)
Rice-wheat rotation under irrigated at different timing, fertilized with a gradient of nitrogen and two rice residue management regimes (removed vs. remained)	Grain nitrogen uptake	India	RMSE: 7.8 kg/ha	Sarkar and Kar (2008)
	Biomass nitrogen uptake	India	RMSE: 8.4 kg/ha	
Rotation systems with fertilization treatment	Soil inorganic nitrogen	United States	Percentage error: < 20%	Hasegawa et al. (2000) ^a
	Soil nitrate	Canada	Absolute error: <50 kg/ha	Beckie et al. (1995) ^b
	Plant nitrogen uptake	United States	Underestimated by 66.7%	Hasegawa et al. (2000)
		Canada	Acceptable for fertilized treatment but heavily underestimated by over 60 kg/ha	Beckie et al. (1995)
Wheat-maize rotation under different nitrogen inputs	Soil nitrogen and nitrogen balance	Hungary	Close to the field measurement with largest disagreement under highest nitrogen input treatment	Kovacs et al. (1995)

(Continued)

Table 19 Summary of the CERES-Wheat model performances for soil nitrogen and plant nitrogen simulations.—cont'd.

Treatment category	Variables	Countries	Performance	References
Soil types	Soil nitrate	France	RMSE: 11.6 ~ 17.8 kg/ha	Gabrielle et al. (2002)
Sowing dates combined with population density	Plant nitrogen content	United States	RMSE: 4.5 mgN/g, Normalized RMSE: 9 ~ 17%	Weiss and Moreno-Sotomayer (2006)
Sowing dates combined with different fertilizer applications	Grain and plant nitrogen uptake	United States	Percentage error: 0.2 ~ 20%	Bacsi and Zemankovics (1995)

^aMaize-wheat rotation followed by legume cover crop, wheat was unfertilized.

^bTreatments included five wheat-fallow rotations and continuous wheat fields under fertilized versus unfertilized treatments.

distribution of nitrate–nitrogen were well-simulated, with absolute errors mostly under 50 kg/ha for five wheat rotation and continuous cropping systems in two locations in Canada. In conventionally managed wheat fields in France, the RMSEs for soil nitrate simulations ranged from 11.6 to 17.8 kg/ha, depending on soil type (Gabrielle et al., 2002). Soil nitrogen balance and accumulative nitrate leaching were acceptably well-simulated (Kovacs et al., 1995).

3.9 Plant Nitrogen Uptake and Plant Nitrogen Content

3.9.1 CERES-Maize

Seven studies have validated the crop nitrogen uptake or crop nitrogen content of the CERES-Maize model given irrigation and fertilization treatments in both continuous cropping and rotation systems (Table 20). Two studies indicated that the simulated nitrogen uptake was highly correlated with observations for nitrogen application treatments (0 ~ 200 kg/ha) in Thailand and nitrogen application (0 ~ 360 kg/ha) combined with irrigation (20 ~ 100 mm) treatments in the United States. The regression lines between the simulations and the observations had R^2 values over 0.9, constants of 0, and respective slopes of 1.1103 and 1.013 (Asadi and Clemente, 2003; Pang et al., 1997). Another study involving a 20 ~ 280 kg/ha nitrogen input gradient with a water deficit in the United States also showed that simulated and the observed nitrogen uptakes were not significantly different ($P \leq 0.05$), but the difference could reach 70 kg/ha for high levels of nitrogen application (about 280 kg/ha) (Pang et al., 1998). The simulations for leaf nitrogen and vegetative nitrogen content in an experiment with 0 ~ 400 kg N/ha applications in China were in agreement with the measurements, with average normalized RMSEs of 23.1 and 24.7%, respectively (Liu et al., 2012). The study of a wheat-maize rotation field with LCC incorporation systems showed that most of the simulated maize nitrogen uptakes were within 20% error in 1 year, but they were underestimated by 25 ~ 70 kg/ha in another year (Hasegawa et al., 2000). For rain-fed maize in Croatia, the grain nitrogen content and nitrogen uptake were overestimated by 30 and 14%, respectively (Vucetic, 2011). Lizaso et al. (2011) tested nitrogen content in shoots, leaves, and stems for varied treatments in Florida and Iowa, US. The RMSEs for shoot nitrogen ranged from 10.2 to 32.6 kg N/ha, with an index of agreement from 0.63 to 0.978. The leaf nitrogen concentration in the percentage simulation had small RMSEs below 0.5% for the 0 and 56 kg N/ha application treatments but large

Table 20 Summary of the CERES-Maize model performances for crop nitrogen uptake and crop nitrogen content variable simulations.

Treatment category	Variables	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized only	Grain nitrogen content	Croatia	Overestimated by 30%	Vucetic (2011)
Well irrigated and fertilized with a gradient of fertilizer(s) only	Nitrogen uptake	Croatia	Overestimated by 14%	Vucetic (2011)
	Nitrogen uptake	Thailand	R^2 : 0.99	Asadi and Clemente (2003)
	Leaf and vegetative nitrogen content	China	Normalized RMSE: 23 ~ 24.7%	Liu et al. (2012)
	Shoot nitrogen content	United States	Index of agreement: 0.70 ~ 0.97	Lizaso et al. (2011)
	Leaf nitrogen content		Index of agreement: 0.74 ~ 0.91	
	Stem nitrogen content		Index of agreement: 0.43 ~ 0.93	
Well irrigated and well fertilized	Shoot nitrogen content	United States	Index of agreement: 0.978	Lizaso et al. (2011)
	Leaf nitrogen content		Index of agreement: 0.614	
	Stem nitrogen content		Index of agreement: 0.817	
Irrigated with a gradient of water and fertilized with a gradient of fertilizer(s)	Nitrogen uptake	United States	R^2 : 0.946	Pang et al. (1997)
		United States	Not significantly different from the observed ($P \leq 0.05$); Difference: up to 70 kg/ha	Pang et al. (1998)

Rain-fed/nonirrigated, well fertilized	Shoot nitrogen content	United States	Index of agreement: 0.632	Lizaso et al. (2011)
	Leaf nitrogen content		Index of agreement: 0.479	
	Stem nitrogen content		Index of agreement: 0.0.638	
Maize-wheat rotation with legume cover crop	Nitrogen uptake	United States	Percentage error: <20%	Hasegawa et al. (2000)
Soil types combined with different irrigation treatments	Soil nitrogen	Bulgaria	R^2 : 0.38 ~ 0.45	Popova and Kercheva (2005)
Wheat-maize rotation under different nitrogen inputs	Soil nitrogen and soil nitrogen balance	Hungary	Close to the field measurement with largest disagreement under highest nitrogen input treatment	Kovacs et al. (1995)

RMSEs of above 0.8% for high nitrogen inputs (above 116 kg N/ha application). Regarding stem nitrogen concentration, in the percentage simulation, the RMSEs were 0.521 ~ 1.45% (Lizaso et al., 2011).

3.9.2 CERES-Wheat

Eight studies validated the nitrogen uptake variable of the CERES-Wheat model (Table 19). Given various population density combined with sowing date treatments in the United States, normalized RMSEs for two wheat cultivars' grain nitrogen concentration ranged from 9 to 17%, and the average RMSE was 4.5 mg N/g (Weiss and Moreno-Sotomayer, 2006). Across low- and high-nitrogen-input treatments in Arizona, the average normalized RMSE for grain nitrogen content was 10.9% (Thorp et al., 2010b). The average normalized RMSE for canopy nitrogen content was 20% across various water and nutrient input rates in China (Ji et al., 2014). Across sowing dates in late-Sep. and early- and late-Nov. in Germany, combined with large fertilizer applications (about 230 kg N/ha) to wheat sown in Sep. and mid-Nov. and 0 versus 213 kg N/ha fertilizer input for wheat sown in late-Nov., the percentage errors for crop nitrogen uptake were within 20%, and the grain nitrogen uptake was overestimated by 0.2 ~ 19.6% (Bacsi and Zemankovics, 1995). The simulated nitrogen uptake for wheat in a fallow-wheat rotation was within 20% error, but it was only about one-third of the observed midwinter nitrogen uptake rate seen for the unfertilized wheat (Hasegawa et al., 2000). Beckie et al. (1995) indicated that crop nitrogen uptake simulations were acceptable for fertilized wheat but that the model heavily underestimated nitrogen uptake for unfertilized wheat by over 60 kg N/ha. In a wheat-rice rotation field in India, given a rice residue (removed vs. remained) and irrigation treatment, the nitrogen uptakes according to grain and biomass simulations had RMSEs of 7.8 and 8.4 kg/ha, respectively (Sarkar and Kar, 2008). Nonetheless, the simulation of plant nitrogen content in Arizona, US, had a normalized RMSE of 50.7% (Thorp et al., 2012).

3.9.3 CERES-Rice

Three studies in Iran and India tested nitrogen content and nitrogen uptake given a fertilization treatment (Table 21). In Iran, the nitrogen content in grains and final biomass were simulated across nitrogen inputs between 0 and 75 kg/ha combined with various levels of irrigation. The RMSEs were both 9 kg/ha, and normalized RMSEs were 12 and 20%, respectively (Amiri et al., 2013). In India, when three rice cultivars were given higher-nitrogen-input treatments (0 ~ 150 kg/ha), the simulated nitrogen uptake by the grains matched reasonably well with the measurement for those

Table 21 Summary of the CERES-Rice model performances for plant nitrogen simulations.

Treatment category	Variables	Countries	Performance	References
Irrigated with varied amount of water and fertilized with varied nitrogen levels	Nitrogen in final biomass Nitrogen in grain	Iran	RMSE: 9 kg/ha Normalized RMSE: 20% RMSE: 9 kg/ha Normalized RMSE: 12%	Amiri et al. (2013)
Well irrigated and fertilized with a gradient of nitrogen inputs	Nitrogen uptake	India	Percentage error: <25% for nitrogen input under 100 kg/ha; Percentage error: 27 ~ 68% for nitrogen input of 100 ~ 150kg/ha	Swain and Yadav (2009)
Wheat-rice rotation, rain-fed, fertilized at varied nitrogen rates, residue managements and planting methods	Nitrogen in grain	India	RMSE: 3.59 ~ 12.44 kg/ha	Sarkar and Kar (2008)

nitrogen addition treatments that were less than 100 kg/ha, with less than 25% error, whereas given high nitrogen inputs of 100 and 150 kg N/ha, the errors were 27 ~ 68% (Swain and Yadav, 2009). Another study in India tested the grain and biomass nitrogen content for transplanted and direct-seeded rice given wheat residue and nitrogen availability treatments. The results showed that the average RMSEs for transplanted and direct-seeded rice grain nitrogen were 12.44 and 3.59 kg/ha, respectively, and that those for biomass nitrogen were 20.78 and 15.38 kg/ha, respectively (Sarkar and Kar, 2008).

3.10 Soil Temperature

Soil temperature variable validation results have been reported only for the CERES-Maize and CERES-Wheat models, not for the CERES-Rice model (Table 22).

3.10.1 CERES-Maize

Four studies have validated soil temperature in the CERES-Maize model. Hasegawa et al. (2000) monitored and simulated soil temperature in a wheat-maize rotation with legume cover crop (LCC) systems. The authors showed that the simulated temperatures did not match well with the observations from Jul. to harvest for maize-LCC rotation and that over half of the simulated temperatures were 2.6 °C different from the observations; some differences were over 10.0 °C (Hasegawa et al., 2000). Under three tillage method treatments, the accuracy of the soil temperatures in a three-layer (0–5 cm, 5–15 cm, and 15–30 cm) simulation varied greatly over various years, layers, and tillage methods, with normalized RMSEs ranging between 22.5 and 49.6%. The results indicated that soil temperature was best simulated under conventional tillage treatment (Liu et al., 2013). Across eight fertilized versus unfertilized combined with irrigated versus unirrigated treatments, the CERES model did not reproduce soil temperature at depths of 5, 10, 20, 40, and 60 cm, with RMSEs of 4.28 °C, 5.5 °C, 6.17 °C, 6.02 °C, and 3.76 °C, respectively (Sandor and Fodor, 2012). Hodges and Evans (1992) tested soil temperature with 4-year field experiments involving row spacing treatments and ten hybrids and reported differences as large as 10 °C and as small as 1 ~ 2 °C.

3.10.2 CERES-Wheat

Hasegawa et al. (2000) also tested the simulated soil temperature of the CERES-Wheat model in wheat-maize rotation with legume cover crop (LCC) systems. The simulated soil temperature in the 0–15 cm and

Table 22 Summary of the CERES-Maize and CERES-Wheat model performances for soil temperature simulations.

Treatment category	Performance	Countries	Reference	Performance	Countries	Reference
	CERES-maize			CERES-wheat		
Irrigated with a gradient of water and fertilized with a gradient of fertilizer(s) only	RMSE: 4.7 ~ 6.2°C	Hungary	Sandor and Fodor (2012)			
Maize-wheat rotation with legume cover crop	Close to the observed through the mid-Aug. but overestimated by up to 10°C in the late season	United States	Hasegawa et al. (2000)	Overestimated by up to 10.7°C, 13.8 °C for 0–15 cm and 15–30 cm, respectively	United States	Hasegawa, et al. (2000)
Soybean-maize rotation with different management intensity ^a	Normalized RMSE: 22.5% ~ 49.6%	China	Liu et al. (2013)			
	RMSE: 3.8 ~ 7°C	China	Liu et al. (2013)			
Row spacing	Difference: 1 ~ 10°C	United States	Hodges and Evans (1992)			

^aManagement intensity included conventional tillage, reduced tillage, and nontillage

15–30 cm layers tended to be higher than those measured by up to 13.8 and 10.7 °C, respectively (Hasegawa et al., 2000).

3.11 Soil Water Content

3.11.1 CERES-Maize

Seventeen papers have tested the soil water validity of the CERES-Maize model, and the tests have been conducted with different soils, water, and nutrient treatments (Table 23). Gabrielle et al. (1995) reported that the mean square error for a soil water storage simulation in three locations (one location grew maize, and other two were bare soils) in France ranged between 4 and 12 cm². The results indicated that soil water storage was most accurately simulated for well-drained soil among these three locations in France. In Bulgaria, both soil water content and potential extractable soil water were reasonably well-simulated for both irrigated and dry plots, with normalized RMSEs within 4 and 19%, respectively (Popova and Kercheva, 2005). In Brazil, the normalized RMSEs for simulating soil moisture across rain-fed and irrigated treatments were within 15% (Soler et al., 2007). In Florida, US, the normalized RMSE for soil water content simulation during the early growing season for the whole profile, 0–5 cm, and 5–15 cm were 35.5, 51.0, and 17%, respectively (Ritchie et al., 2009). Asadi and Clemente (2003) reported that with four levels of nitrogen application from 0 to 200 kg/ha in Thailand, the simulated soil water content was reasonably well-simulated, with some notable underestimations of up to 20% error. Liu et al. (2014) reported that the normalized RMSEs for soil water content at the 0–10 cm layer under unfertilized and fertilized treatments in Canada ranged between 25 and 36%. With 119 data points for four types of irrigated soils in Georgia, US, Hook et al. (1994) calculated that the model underestimated soil water content by an average of 5.92 mm and that the accumulative absolute difference was 16.66 mm (Hook, 1994). In another US state, soil moisture was tested in four layers (0–120 cm with 30 cm intervals) on irrigated land as well. The results indicated that soil moistures were generally well-simulated for each layer, with normalized RMSEs under 14.0%, except for the top, 0–30 cm, layer (normalized RMSE of 16.6%) (Xevi et al., 1996). Anothai et al. (2013) showed that given 70%– and 100%–full irrigation treatments, the soil water content simulation using the Priestley–Taylor approach to ET estimation performed reasonably well (normalized RMSE of 13.2 ~ 29.0% for 0 ~ 15 cm) in 1 year but poorly in

Table 23 Summary of the CERES-Maize model performances for soil water and plant extractable soil water (PESW) simulations.

Treatment category	Variables	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized	Soil water content	United States	Percentage error: >10%	DeJonge et al. (2011)
		United States	RMSE: 0.043 m ³ /m ³	Saseendran et al. (2008)
Irrigated with a gradient of water and well fertilized	Soil water content	Spain	Not accurate	López-Cedrón et al. (2008)
		Brazil	Normalized RMSE: <15%	Soler et al. (2007)
	Soil water content, 0–15 cm Soil water content, 15–45 cm Soil water content, 45–75 cm Soil water content, 75–120 cm Soil water storage	United States	Percentage error: <8%	DeJonge et al. (2011); Jara and Stockle (1999)
		United States	RMSE: 0.025 m ³ /m ³	Saseendran et al. (2008)
		United States	Normalized RMSE: 13.2 ~ 58.4%	Anothai et al. (2013)
			Normalized RMSE: 12.7 ~ 44.7%	
			Normalized RMSE: 17.2 ~ 29.3%	
			Normalized RMSE: 9.2 ~ 25.0%	
		China	Difference: <27 mm	Yang et al. (2006a)
		Thailand	Underestimated by up to 20%	Asadi and Clemente (2003)
	Soil water content			
Well irrigated and fertilized with a gradient of fertilizer(s)				

(Continued)

Table 23 Summary of the CERES-Maize model performances for soil water and plant extractable soil water (PESW) simulations.—cont'd.

Treatment category	Variables	Countries	Performance	References
Well irrigated and well fertilized	Soil water content, whole profile	Bulgaria, United States	Normalized RMSE: 4%, 35.5%	Popova and Kercheva (2005); Ritchie et al. (2009)
	Soil water content, 30 cm-layers from soil depth of 30–120 cm	United States	RMSE: $0.064 \sim 0.073 \text{ m}^3/\text{m}^3$	Saseendran et al. (2005)
		United States	Normalized RMSE: <14%	Xevi et al. (1996)
	Soil water content, 0–5 cm	United States	Normalized RMSE: 51%	Ritchie et al. (2009)
	Soil water content, 5–15 cm	United States	Normalized RMSE: 17%	Ritchie et al. (2009)
	Soil water content, 0–30 cm	United States	Normalized RMSE: 16.6%	Xevi et al. (1996)
	PESW	Bulgaria	Normalized RMSE: 19%	Popova and Kercheva (2005)
	Soil water content, 0–10 cm	Canada	Normalized RMSE: 30 ~ 35%	Liu et al. (2014)
Not fertilized	Soil water content, 0–10 cm	Canada	Normalized RMSE: 25 ~ 36%	Liu et al. (2014)
Soil types	Soil water storage	France	Mean square error: $4 \sim 12 \text{ cm}^2$	Gabrielle et al. (1995)
		United States	Average underestimation: 5.92 mm	Hook (1994)
Management intensity ^a	Soil water content in each layers	United States	Average RMSE: $0.042 \sim 0.054$	Garrison et al. (1999)
		China	RMSE: $0.01 \sim 0.11$	Liu et al. (2013)

^aManagement intensity included conventional tillage, reduced conventional tillage, and no-tillage.

another year (normalized RMSE of 49.4 ~ 58.4% for 0 ~ 15 cm). The authors also showed better simulations for deeper soils during both of the growing seasons, with normalized RMSEs of 12.7 ~ 44.7%, 17.2 ~ 29.3%, and 9.2 ~ 25% for 15–45 cm, 45 ~ 75 cm, and 75 ~ 120 cm soils, respectively (Anothai et al., 2013). Most of the study results suggested that the simulated soil water content was acceptably matched with the observations, but the model accuracies varied among the studies. Two studies in Colorado, US, with a range of irrigation water treatments showed that soil water simulations were more accurate given fully irrigated treatments. One study reported that the relative error for soil water simulations were under 8.0% for full-irrigation treatments and over 10.0% for limited irrigation treatment (DeJonge et al., 2011). The other reported that the average RMSEs were $0.025 \text{ m}^3/\text{m}^3$ and $0.043 \text{ m}^3/\text{m}^3$ for soils irrigated with a 23 ~ 188 mm treatment and a rain-fed treatment, respectively (Saseendran et al., 2008). Another study in Colorado involving well-irrigated and well-fertilized treatments reported average RMSEs of 0.064 ~ 0.073 when simulating soil water for three maize cultivars (Saseendran et al., 2005). In Iowa, US, the average RMSEs ranged from 0.042 to $0.054 \text{ cm}^3/\text{cm}^3$ across both tilled and no-till experiments in two types of soils in Iowa (Garrison et al., 1999). Garrison et al. (1999) also reported that the simulated soil storage was in good agreement with the measurement. A study in China also showed that given four and five irrigations per growing season, the overall errors for soil water storage were under 27.0 mm (Yang et al., 2006a). Under various management intensities, Liu et al. (2013) reported a wide range of RMSEs (from 0.01 to 0.11) for 0–20 cm and 0–30 cm soil water content simulations for various years under conventional tillage, reduced conventional tillage, and nontillage treatments. They also pointed out that the worst simulation may be due to inaccurate soil water content measurement caused by following the gravimetric water content measurement protocol (Liu et al., 2013). Jara and Stockle (1999) reported that the average RMSE for soil moisture simulation across full irrigation, partial irrigation, and no irrigation treatments in California, US, was $0.016 \text{ m}^3/\text{m}^3$, with relative error of 7.64%. In addition, the authors noted that the CERES model tended to underestimated soil moisture in the top 15 cm for field experiments in both Washington State and California. However, a graphical comparison between simulated and measured soil water performed by López-Cedrón et al. (2008) indicated a relatively poor simulation (López-Cedrón et al., 2008).

3.11.2 CERES-Wheat

Twelve studies validated the soil water variable under various soils, water availability treatments, fertilization treatments, and management intensity treatments (Table 25). For low-permeability Vertisol soils in Bulgaria, soil water content was more accurately simulated when the soil was fertilized with a 200 kg N/ha treatment than when an unfertilized treatment was applied, with normalized RMSEs of 1.3 and 4.8%, respectively (Popova and Kercheva, 2005). The potentially extractable soil water simulation was also more accurate given a fertilized treatment (normalized RMSE = 13.3%) than an unfertilized treatment (normalized RMSE = 36.8%). With fertilized moderately permeable Chromic Luvisol soils, the average normalized RMSE for soil water content and potentially extractable soil water were 1.6 and 16.25%, respectively (Popova and Kercheva, 2005). A simulation study in Austria tested the soil water content of three soils for both the whole soil profile and at three soil depths (0–30, 0–60 and 0–90 cm). The results indicated that the soil moisture content simulations were least accurate for sandy Chernozem soil, with normalized RMSEs of 7.0, 3.8, 6.2, and 8.6% for whole soil profile moisture content and soil water content at depths of 30, 60, and 90 cm, respectively, whereas those for Chernozem and fluvisol soils were within 4.0, 1.1, 4.6, and 6.5%, respectively (Eitzinger et al., 2004). In Argentina, across full irrigation and early drought treatments, the simulated soil water content was correlated with the observations ($R^2 = 0.86$) (Savin et al., 1994). With clay and silt soils in Canada, the RMSE for soil water content at varied layers ranged between 0.025 and 0.046 (He et al., 2014). However, the simulated water content for both full-irrigation and full-drought treatments in New Zealand did not match well with the observations, with some heavy underestimation (Jamieson et al., 1998). Given 0 ~ 4 irrigations during the growing season, the differences between the simulated and observed soil water storages were within 3% for 0 ~ 2 irrigations, and the largest difference was 56 mm, which occurred given four irrigation treatments (Yang et al., 2006a). For two-fertilizer input (110.5 and 241.0 kg N/ha) treatments in Arizona, the soil water content validation showed that the model simulated deeper soil layers (normalized RMSE of 3.3 ~ 8.7% for 30–120 cm soil layers) better than the top soil layer (normalized RMSE of 18.9% for 0–30 cm soil) (Thorp et al., 2010b). The other soil water test under tillage methods, including conventional moldboard plowing 40–45 cm deep, ripper subsoiling at 60–70 cm deep, surface disc-harrowing at 15–20 cm deep, and minimum tillage with rotary hoeing treatment, was conducted in a farm in Italy. The revised CERES model, in which the evapotranspiration equation

was changed and calibrated for this farm, simulated soil water fairly well, with an average standard error of regression of $0.03 \text{ cm}^3/\text{cm}^3$. The simulated soil water was particularly accurate for all soil layers under the ripper subsoiling treatment, except for the 20–40 cm layer, with a linear regression slope that was not statistically different from 1 ($P < 0.1$). The authors also pointed out that under minimum tillage treatment, the simulated soil water was close to the measurement except for those in a drought period (Castrignano et al., 1997). The simulated soil water storage and content in various layers were in good agreement with the measured values for long-term agricultural experiment sites in Canada for both continuous wheat and wheat rotation cropping systems (Beckie et al., 1995). In another location in Canada, the simulated soil water content was consistently smaller than the measured value for the surface layers, but it was consistently larger than the measured value for deep soils (Wang et al., 2010). Under fertilization treatments of $0 \sim 112 \text{ kg/ha}$ nitrogen addition for 3 years, the RMSEs for soil profile moisture were within 0.074 (Saseendran et al., 2004). By comparing simulated water stress and water moisture monitored by sensors, Povilaitis et al. (2010) found that both the simulated water stress and the measured soil water content provided acceptable soil water information regarding conventional field trials with optimum fertilizer applications, integrated field trials with lower and organic fertilizer application, and no fertilizer application field trials (Povilaitis and Lazauskas, 2010).

3.12 Evapotranspiration and Deep Seepage

The evapotranspiration variable has been validated for the CERES-Maize and the CERES-Wheat models (Tables 24 and 25). The deep seepage of the CERES-Wheat model has been evaluated as well. No research has reported on the ET or deep seepage validation for the CERES-Rice model.

3.12.1 CERES-Maize

Two studies tested crop water use. Yang et al. (2006) simulated maize water use under no irrigation versus two irrigations per growing season and reported that water use was overestimated by 42.7 mm (0.418 mm/day) and 67.4 mm (0.661 mm/day), respectively (Yang et al., 2006a). The other study, in Washington, US, reported that the plant water use simulation had normalized RMSEs of 8.95 and 6.98% for limited and full irrigation, respectively (RMSEs of 0.33 and 0.27 mm/day, respectively) (Jara and Stockle, 1999).

Table 24 Summary of the CERES-Maize model performances for crop water use and evapotranspiration (ET) simulations.

Treatment category	Variables	Countries	Performance	References
Rain-fed/nonirrigated and well fertilized	ET	United States	Percentage error: >12%	DeJonge et al. (2011); DeJonge et al. (2012)
Irrigated with a gradient of water and well fertilized	ET	Italy, Turkey, Pakistan	Normalized RMSE: <10% Percentage error: 2.3 ~ 7.64% R^2 : 0.78	Anothai et al. (2013) Mastrorilli et al. (2003); Ben Nouna et al. (2000); Gercek and Okant (2010); Mubeen et al. (2013)
	Crop water use	United States	RMSE: 0.27 ~ 0.33 Normalized RMSE: 7 ~ 9%	Jara and Stockle (1999)
		China	Overestimated by 42.7 ~ 67.4 mm	Yang et al. (2006)a
Sowing date	ET	United States	RMSE: 3.7 ~ 5.1 cm	Saseendran et al. (2005)

Table 25 Summary of the CERES-Wheat model performances for soil water, plant extractable soil water (PESW) and evapotranspiration (ET) simulations.

Treatment category	Variables	Countries	Performance	References
Rain-fed and well fertilized	Soil water loss	China	Error: 1 cm	Yang et al. (2006b)
Irrigated with a gradient of water/different scheduling time and well fertilized	Soil water loss Soil water content	China	Error: 13 ~ 43 cm	Yang et al. (2006b)
		Argentina	R^2 : 0.86	Savin et al. (1994)
		New Zealand	Did not match well	Jamieson et al. (1998)
		China	Percentage error: <3% difference up to 56 mm	Yang et al. (2006a)
Well irrigated and fertilized at varied rates	ET	United States	Normalized RMSE: <10% for layers below 30cm, 18.9% for 0–30 cm layer	Thorp et al. (2010b)
		New Zealand	Difference: 9 ~ 60 mm	Jamieson et al. (1998)
		United States	Normalized RMSE: 2.4%	Thorp et al. (2010b)
		United States, China	Normalized RMSE: <3%	Kang et al. (2009)
Irrigated and fertilized at varied rates	ET	India, China	RMSE: 25 mm Normalized RMSE: 4 ~ 9%	Arora et al. (2007) Ji et al. (2014)
Rain-fed and varied amount of fertilizer(s)	Soil water content	United States	RMSE: <0.074	Saseendran et al. (2004)
	ET	United States	RMSE: 9.2 ~ 13.5 mm	Saseendran et al. (2004)

(Continued)

Table 25 Summary of the CERES-Wheat model performances for soil water, plant extractable soil water (PESW) and evapotranspiration (ET) simulations.—cont'd.

Treatment category	Variables	Countries	Performance	References
Soil types	Soil water content	Bulgaria, Australia, Canada	Normalized RMSE: 1.3 ~ 9% RMSE: 0.025 ~ 0.046	Popova and Kercheva (2005) ^b ; Eitzinger et al. (2004); He et al. (2014)
	PESW	Bulgaria	Normalized RMSE: 13.3 ~ 36.8%	Popova and Kercheva (2005)
Tillage methods ^c	ET	Australia	Overestimated by 61 ~ 87 mm	Eitzinger et al. (2004)
	Soil water	Italy	Standard error of regression: 0.03	Castrignano et al. (1997)
Wheat-fallow and continuous wheat under different fertilization rate	Soil water levels	Canada	Accurate for 0–30 cm layers with <1 m error, significantly different for most 60–150 cm layers (P=0.05, 0.01)	Beckie et al. (1995)
	Soil water content	Canada	$R^2 < 0.64$	Wang et al. (2010)
CO ₂ concentrations with two irrigation regimes	ET	United States	Normalized RMSE: <14%	Tubiello et al. (1999b)
Planting densities combined with varied fertilization rates	ET	United States	Difference: 19 ~ 33 mm	Thorp et al. (2010a)

^aLiteratures that did not include treatments were considered as “well irrigated and well fertilized”;

^bTreatments included soil types combined with different fertilization rate treatments.

^cIncluding conventional moldboard ploughing, ripper subsoiling, surface disc-harrowing, and minimum tillage with rotary hoeing.

For ET simulation validation, two studies in Colorado, US, reported ET simulations that had percentage errors under 7.5% for full-irrigation treatment and over 12.0% for limited irrigation treatment (DeJonge et al., 2011, 2012). When simulating ET given three levels of irrigation water treatments, from nonwater-stress to severe water stress, the model predicted seasonal ET well for all three levels, with zero error under nonwater-stress conditions, up to 3.5% underestimation under moderate water stress conditions, and up to 7.64% under severe water stress condition (Ben Nouna et al., 2000; Mastrorilli et al., 2003). A similar ET simulation for a 2-year experiment with three levels of irrigation, 100%-, 75%-, and 50%-full, showed that the percentage errors were about 3.0%, 2.3%–5.1%, and above 3.7%, respectively (Gercek and Okant, 2010). In Pakistan, across seven irrigation treatments of varying amounts and timings, the coefficients of determination between the simulated and observed ETs were above 0.78 (Mubeen et al., 2013). Across six irrigation treatments in Colorado, US, the average normalized RMSE for ET simulation was within 10%, with an index of agreement above 0.9 (Anothai et al., 2013). With well-irrigated and sowing date treatments, the ET simulations had RMSEs of 3.7 ~ 5.1 cm, depending on the hybrids involved (Saseendran et al., 2005).

3.12.2 CERES-Wheat

Ten studies tested the ET of the CERES-wheat model under varying water availability treatments, CO₂ concentrations, nutrient application treatments, and soils (Table 25). Arora et al. (2007) tested the seasonal ET with 5-year data collected from field experiments with varying water regimens (eg, irrigation timing at irrigation water-to-pan evaporation ratios of 1.2, 1.0, and 0.6 after sowing and 1–2 irrigations after sowing) combined with nutrient application regimens (eg, 0–180 kg N/ha, 120 kg N/ha applied at various timings, etc.). They reported that across all the field treatments, the simulated ET had an average RMSE of 25 mm, equivalent to a 9% normalized RMSE (Arora et al., 2007). Yang et al. (2006b) showed that the simulated soil water loss was close to that observed under no irrigation treatment (error: 1 cm), but the simulation did not match well with the observations given a four-irrigation treatment (error: 43 cm). A similar study in China in which wheat fields were irrigated zero to four times showed that the soil water content was mostly well-simulated (percentage error: <3%), but at the end of the growing seasons, the soil water simulation errors ranged up to 56 mm (Yang et al., 2006a). Across nine treatments with various levels of nutrient and water input in China, the normalized RMSE for the ET simulation was

between 4 and 7% (Ji et al., 2014). By contrast, Jamieson et al. (1998) reported that given weekly irrigation treatment, the total ET was underestimated by 135 mm, while under no irrigation treatment, it was overestimated by 60 mm. The authors also indicated that for total ET simulations under early drought and late drought treatments, the absolute errors mostly ranged from 9 to 17 mm, with one exception of 51 mm (Jamieson et al., 1998). When simulating cumulative ET under treatments combining CO₂ concentration (ambient vs. 550 ppm) and irrigation (well-watered vs. water deficit), the normalized RMSEs were within 14% (Tubiello et al., 1999b). With nutrient applications of 0 ~ 112 kg N/ha for 3 years, the simulated seasonal ET had RMSEs from 9.2 to 13.5 cm (Saseendran et al., 2004). With 110.5 and 241.0 kg N/ha application treatments, the normalized RMSE was 2.4% (Thorp et al., 2010b). The authors also noticed that the model did not simulate deep seepage, as opposed to the approximately 30 mm of seepage that were measured (Thorp et al., 2010b). Another study in Arizona, with various nitrogen input and population density treatments, showed that the difference between the simulated and observed ET varied from 1.9 to 3.3 cm given a sparse planting and high nitrogen input treatment (Thorp et al., 2010a). One study in Austria tested ET with field experiments on three types of soils. The results indicated that the model overestimated the seasonal ET for the three types of soil by 61 ~ 87 mm (Eitzinger et al., 2004). Kang et al. (2009) used multiple seasons of wheat field data from two sites in China and the United States to validate the ET calculated by the Priestley–Taylor and Penman equations. The authors reported that the average RMSEs were 2.1 and 2.5 mm (these are either mm/day or mm/season), respectively (Kang et al., 2009).

3.13 Other Variables

3.13.1 CERES-Maize

Rezzoug et al. (2008) reported that the simulation of number of years per square meter the variable had an RMSD of 74.21 and a mean absolute percentage error of 29.66%. Popova et al. (2005) compared the simulated and the measured accumulative drainage graphically, and the results indicated that the simulated drainage was underestimated for an irrigated maize field in Bulgaria (Popova and Kercheva, 2005). Lizaso et al. (2001, 2003a,b) used two datasets from different sites in Iowa with different treatments to test the intercepted photosynthetically active radiation (IPAR) variable. Comparing the simulated IPAR to the

observed IPAR under two extreme nitrogen rates (0 vs. 224 kg N/ha) combined with different maize hybrids, planting dates, and population density treatments, the authors reported that the IPAR was overestimated, especially for the nonnitrogen application treatment (Lizaso et al., 2001). Using 72 data points from experiments on irrigated maize fields with varying population densities, hybrids, and nitrogen application rates (56 vs. 168 kg N/ha) and nonirrigated maize fields with varying population densities and hybrids, the authors also found that IPAR was overestimated, with a mean error of 0.41 MJ/plant and an RMSE of 0.75 MJ/plant (Lizaso et al., 2003b). In Australia, Carberry (1991) tested the thermal time of the CERES-Maize model and showed that the observed temperature for leaf tip appearance was 48.3 °C, as opposed to the 38.9 °C simulated by the model. Two studies compared simulated irrigation water use to historical irrigation water use records. Salazar et al. (2012) reported that the simulated monthly irrigation water use was in close agreement with the historical record for the South Georgia, US, region, but there was a notable overestimation for early growing seasons when rainfall was not abundant. The study of a wheat-maize rotation field in the North China Plain showed that the simulated highest and lowest water use of the irrigated wheat matched with the records, and for the 5-year simulation, the average difference was 12 mm. When simulating the rotation irrigation water use by two crops, the average difference was 6.1 mm, and the absolute differences for each year ranged between 0 and 69 mm (Yang et al., 2006a).

Two studies in the United States and Turkey calculated and tested the water productivity variable. DeJonge et al. (2012) reported that the simulation was reasonably accurate for full irrigation (RMSD of 3.45 kg/ha per mm and underestimated by 6.53%), but much less accurate for limited irrigation (RMSD of 5.97 kg/ha per mm and underestimated by 26.7%) in a water-use efficiency simulation. By contrast, the similar study in Turkey found that water use efficiency was under-predicted by 1.5% under full irrigation conditions while it was overpredicted by 1.4 and 1.7% for 75%- and 50%-full irrigation treatments, respectively (Gercek and Okant, 2010).

3.13.2 CERES-Wheat

Zhang et al. (2012) compared the simulated and observed cumulative frequency distributions of the optimal nitrogen rate in Oklahoma, US, for 37 years and reported that the optimum nitrogen application rates were well-simulated when nitrogen application was under 67 kg/ha, underestimated for 67 and 90 kg N/ha treatments, and overestimated

for 90 and 112 kg N/ha treatments. Water use efficiency and nitrogen partial factor productivity were validated with varied water and nitrogen input treatments in China, and the normalized RMSEs were 5 ~ 8 and 5 ~ 6%, respectively (Ji et al., 2014).

3.13.3 CERES-Rice

With a fertilization treatment of 0 ~ 150 kg/ha at various timings in Thailand, Cheyglinted et al. (2001) calculated the grain-straw ratio and reported that the relative absolute percentage errors for the ratio between grain and straw simulations were 21 ~ 32%.

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Impact of Herbicides on Soil Biology and Function

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Abstract

There is a growing awareness among farmers about the importance of soil for sustaining crop production and providing beneficial ecosystem services. Over the last 2 decades, global herbicide use has increased as farmers have shifted to more sustainable conservation tillage practices and have adopted herbicide-tolerant crop cultivars. The implications of increased herbicide use for soil biology are being questioned, but a comprehensive review on this topic is lacking. In this chapter we outline the chemistry and use of the major herbicide classes, and review the soil functions relevant to crop production. We then collate and critically evaluate the evidence for herbicide effects on soil biota and activity. In general, most studies suggest that the impacts of herbicide application on soil function are only minor and/or temporary. However, there are some instances where findings consistently suggest effects that could significantly alter soil function. These include disruptions to earthworm ecology in soils exposed to glyphosate and atrazine; inhibition of soil N-cycling (including biological N₂-fixation, mineralization and nitrification) by sulfonylurea herbicides in alkaline or low organic matter soils; and site-specific increases in disease resulting from the application of a variety of herbicides. Issues with extrapolating these findings to broadacre farming include the lack of a consistent framework for assessing herbicide risk to soil biology, the relevance of the magnitude of herbicide impacts compared with the impacts of other soil management practices such as tillage or crop rotation, the complexity of herbicide formulations and mixtures, and the limited number of long-term field studies.



1. INTRODUCTION

Weeds are a significant constraint and cost to agricultural production worldwide. Estimates suggest that without weed control, yield losses could range from 29% in wheat to 47% in rice crops (Oerke, 2005). Historically, weed control was achieved manually or mechanically involving some form of soil cultivation. Although this is certainly effective for reducing weed incidence and in providing a suitable seed bed for sowing crops, the mechanical disturbance of soil also has a number of detrimental side-effects, such as increasing the risks of soil erosion (Pimentel et al., 1995) and loss of soil organic matter (Six et al., 1999). Repeated mechanical tillage also incurs

relatively high labor and energy costs which impact on gross farm income (Karlen et al., 2013).

In order to overcome the adverse effects caused by repeated soil cultivation, farmers have increasingly adopted “conservation tillage” practices whereby soil cultivation is minimized. For example, in Australia the proportion of grain growers using no-till technology has increased over the last 30 years from around 5% to over 70% (Llewellyn et al., 2012). The move toward conservation tillage has required the use of alternative weed-control strategies, including a higher reliance on herbicides (D’Emden et al., 2006). The development of herbicide-resistant crop varieties, through conventional breeding or genetic modification (GM), has also promoted herbicide use but in a more selective manner to match the mode of crop-herbicide resistance. Benbrook (2012) estimated herbicide-resistant crop technology led to a 239 M kg increase in herbicide use in the United States between 1996 and 2011, mainly through increased glyphosate use. It is also estimated that herbicide usage in the Australian grains industry increased by more than 30% from 2002 to 2012, from a value of \$700 M to \$1.1 B over that period (APVMA, 2003, 2012; data CPI and land-area adjusted). Although precise data for the total increase in the volume of herbicides applied worldwide are not readily available, herbicide use is predicted to increase as food production intensifies and urbanization continues to put pressure on the availability of labor for manual weed control, especially in developing countries (Gianessi, 2013).

Unfortunately, little is known about the impact of increased herbicide use on soil biota and the ecosystem services they provide. This in part reflects the diversity of the chemicals being applied and also the diversity in soil ecological communities and function, which renders a full systematic assessment almost impossible. A review of the effects of herbicides on soil biology almost a decade ago (Bünemann et al., 2006) suggested that the database of knowledge is “simply too small to draw sound conclusions,” and a more recent review emphasized the lack of a suitable framework for the routine evaluation of pesticide effects on soil microbial communities and functions (Imfeld and Vuilleumier, 2012). Scheepmaker and van de Kastelee (2011) conducted a broad meta-analysis on the effects of chemical control agents (including fungicides, insecticides, herbicides, and antagonists) on a number of nontarget microbial soil organisms by pooling different microbial count variables, such as colony-forming units per gram of soil, nodule score, root colonization, mycorrhizal root length, induced host mortality, and number

of taxa. Unfortunately, herbicides were excluded from the final analysis because of limited data availability. Even the conclusions from the analysis of insecticides and fungicides lacked the resolution to make specific recommendations about individual compounds.

From the few review papers available, the emerging picture is one of compound-specific effects on particular soil functions. With regard to herbicides, adverse effects on phosphatase activity by glyphosate (Sannino and Gianfreda, 2001), inhibition of nitrification by simazine (Hernández et al., 2011) and adverse effects on pathogen-antagonistic *Pseudomonas* bacteria by acetochlor and chlorimuron-ethyl (Wang et al., 2013; Wu et al., 2009) are just some of the examples of the potential effects of herbicides on soil health as related to plant nutrition and disease.

In light of these reports, herbicides applied with the ultimate goal of maximizing productivity and economic returns potentially act at the expense of ecosystem functions. Although not immediately obvious, these ecosystem services also contribute to crop health by promoting of crop stubble turnover, pathogen suppression, nutrient cycling, and maintenance of soil structure. Sandhu et al. (2008) estimated that soil functions provide approximately \$330 per ha per year of unaccounted, nonmaterial value in “conventional” agricultural systems, and up to \$500 per ha in “organic” systems. As an example, this would equate to a value of over \$6 billion per year to Australian (conventional) grain production, compared to the gross value of grain production of around \$9 billion/year.

A comprehensive review of the impacts of herbicides on soil biota and functions is required to gain insight into if, or how, their use should be changed to improve productivity and economic returns. Synthesis of the current knowledge will enable farmers to identify where a change in herbicide management could protect the ecosystem services provided by their soils in crop production. In this paper, we first summarize the chemistry of the different herbicide classes and review their mode of action with regard to potential effects on nontarget organisms. Second, we outline the methods used by researchers to measure herbicide-induced changes to soil biological communities. Subsequently, we evaluate the empirical evidence for direct effects of herbicides on nontarget soil organisms and biological community structure, followed by effects on key ecosystem functions contributing to agricultural production. These functions include carbon (C) turnover, nutrient cycling, and the suppression of disease. We then discuss a number of factors that confound or moderate our understanding of the impacts of herbicides. Finally, we identify priority areas for monitoring and knowledge

gaps that limit our current ability to draw appropriate conclusions on the impact of herbicides on soil biology and function.

2. HERBICIDE CHEMISTRY AND MODE OF ACTION

A chemical is classified as a herbicide if its toxicity toward plants in orders of magnitude is higher than its toxicity toward other organisms. Within this broad grouping, however, are numerous chemical classes with distinct modes of herbicidal action. Thus, the effect of each chemically unique herbicide on nontarget organisms is likely to be different and difficult to generalize. Unfortunately the exact worldwide total usage for each individual or class of chemicals is not known. To help focus our review, we searched the database Scopus using the search terms herbicide AND soil AND (microb* OR function*) and ranked the herbicide classes in terms of the most to least studied. The frequency of study (Fig. 1) roughly correlates

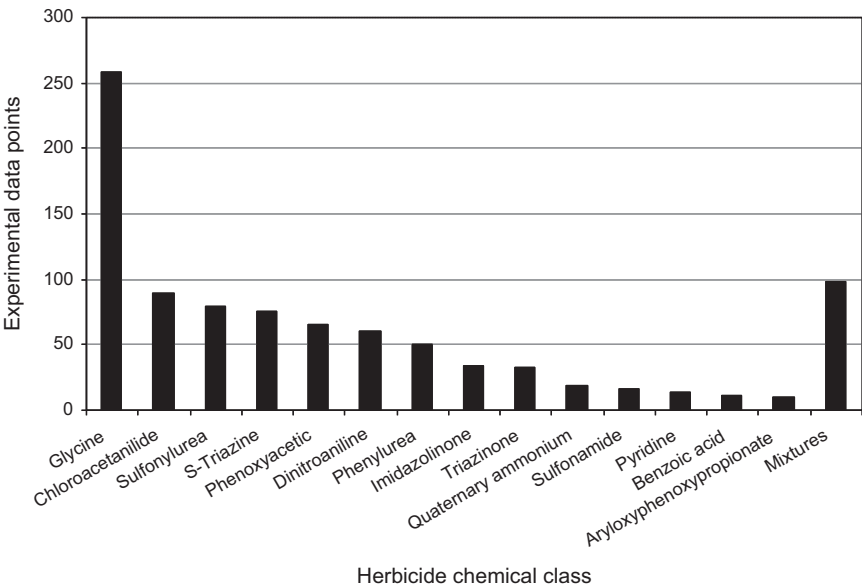


Figure 1 Frequency of experimental studies on different herbicide classes returned from a search of the database Scopus, using the search terms *herbicide AND soil AND (microb* OR function*)*.

to the frequency of use, based on sporadic and localized surveys (Givens et al., 2009; Grube et al., 2011; Osten et al., 2007). There is obviously a strong focus in the literature on the herbicide glyphosate, concomitant with its widespread use.

A further complication in predicting effects of herbicides on soil biology and function is the relationship between herbicide application rate and its concentration in the soil. Most ecotoxicological studies are conducted by constructing dose–response curves with the dose of toxicant reported as a concentration (eg, mg/L or mg/kg). However, in practice, herbicides are applied at rates given in kilogram, or liter per hectare. The herbicide concentration in soil initially depends on the depth of incorporation and the bulk density of the soil. After application, herbicides redistribute through the soil profile and concentrations decrease over time via abiotic and biotic loss and transport pathways. A major issue is the failure of many reports to explain the assumptions used to calculate the herbicide rates used in incubation studies (in mg/kg) from field rates (in g/ha). In order to give the reader some perspective, we have calculated predicted environmental concentrations (PEC) in soil for different model herbicides according to European Economic Community (2007) guidelines (Table 1). In this scenario, a surface-applied herbicide is assumed to be distributed in the top 5 cm of a soil profile with a bulk density of 1.5 g/cm³. Herbicide concentrations reported in this document refer to the concentration of the active ingredient unless otherwise specified. Chemical structures and IUPAC nomenclature for the model herbicides are available for reference in Appendix 1.

2.1 Glycine

Glyphosate is the most widely used herbicide in the world (Duke and Powles, 2008), with more than twice the mass of the next most popular herbicide being applied in the USA (Grube et al., 2011). It is a nonselective herbicide, which is taken up through leaves and shoots and is distributed throughout the plant tissues. Glyphosate is known for its high sorption affinity for soil (especially clays and other minerals) and therefore is considered essentially nonmobile in soil (Table 1). Glyphosate is not considered persistent as it undergoes biotransformation into aminomethylphosphoric acid (AMPA) as its main metabolite.

Glyphosate prevents the synthesis of aromatic amino acids, by binding to the enzyme 5-enolpyruvateshikimate-3-phosphate synthase (EPSPS) and inhibiting its function (Sikorski and Gruys, 1997). Bacteria and fungi require aromatic amino acids for protein synthesis and for the production

Table 1 Major herbicide classes, model compounds, and their application rates.

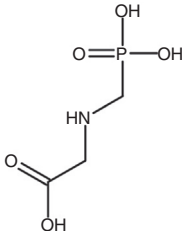
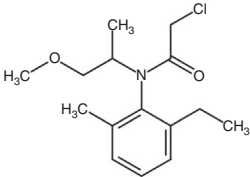
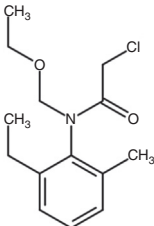
Herbicide class	Mode of action	Example herbicides	Typical label application rate (kg/ha)	Predicted concentration in soil (mg/kg) ^a	Sorption coefficient (K_{oc}) ^b	Persistence (half-life in days) ^b
Glycine	Inhibition of enolpyruvylshikimate-3-phosphate (EPSP) synthase	Glyphosate	2.2	2.9	1435	12
Chloroacetimide	Inhibition of very long-chain fatty acid synthesis (cell division)	Metolachlor	2.9	3.9	120	90
		Acetochlor	5.8	7.7	156	14
Sulfonylurea	Inhibition of acetolactate synthase (branched chain amino acid synthesis)	Chlorsulfuron	0.02	0.027	36.3	160
		Metsulfuron-methyl	0.005	0.007	92	66
Triazine	Inhibition of photosynthesis at PSII	Atrazine	3	4	100	75
		Simazine	2	2.7	130	60
Phenoxy-carboxylic acids	Synthetic auxins	2,4-D	1.1	1.5	4.4	39.3
		MCPA	1.1	1.5	74	24
Ureas, Amides	Inhibition of photosynthesis at PSII	Diuron	1	1.33	813	75.5
		Propanil	6	8	152	0.4
Dinitroaniline	Inhibition of microtubule assembly	Trifluralin	1	1.33	15800	181
		Pendimethalin	1.5	2	17581	90
Imidazolinone	Inhibition of acetolactate synthase (branched chain amino acid synthesis)	Imazethapyr	0.1	0.133	52	90
		Imazamox	0.035	0.047	67	25

Explanations for the modes of action are given in the text.

^aAssuming a bulk density of 1.5 g/cm³ and a herbicide distribution in the top 50 mm of soil (European Economic Community, 2007).

^bSorption and degradation data sourced from PPDDB (2015).

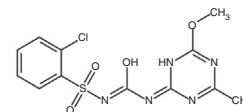
Appendix 1 Chemical structures and IUPAC names of commonly used herbicides.

Herbicide class	Example herbicides	IUPAC name	Chemical structure
Glycine	Glyphosate	2-[(Phosphonomethyl)amino]acetic acid	
Chloroacetimide	Metolachlor	2-Chloro- <i>N</i> -(2-ethyl-6-methylphenyl)- <i>N</i> -(1-methoxypropan-2-yl)acetamide	
	Acetochlor	2-Chloro- <i>N</i> -(ethoxymethyl)- <i>N</i> -(2-ethyl-6-methylphenyl)acetamide	

Sulfonylurea

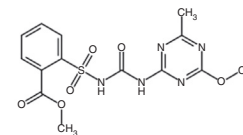
Chlorsulfuron

N'-(2-chlorobenzenesulfonyl)-*N*-(6-methoxy-4-methyl-1,2-dihydro-1,3,5-triazin-2-ylidene) carbamimidic acid



Metsulfuron-methyl

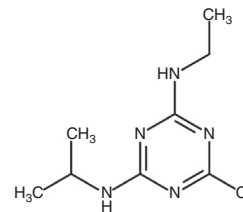
Methyl 2-({[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) carbamoyl]amino}sulfonyl) benzoate



Triazine

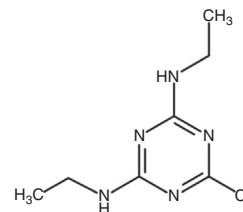
Atrazine

6-Chloro-*N*2-ethyl-*N*4-(propan-2-yl)-1,3,5-triazine-2,4-diamine



Simazine

6-Chloro-*N*2,*N*4-diethyl-1,3,5-triazine-2,4-diamine

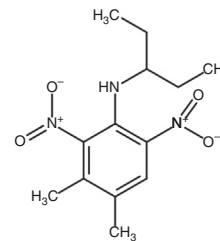


(Continued)

Appendix 1 Chemical structures and IUPAC names of commonly used herbicides.—cont'd.

Herbicide class	Example herbicides	IUPAC name	Chemical structure
Phenoxycarboxylic acids	2,4-D	2-(2,4-Dichlorophenoxy)acetic acid	
	MCPA	2-(4-Chloro-2-methylphenoxy)acetic acid	
Ureas, amides	Diuron	1-(3,4-Dichlorophenyl)-3,3-dimethylurea	
	Propanil	N-(3,4-Dichlorophenyl)propanimidic acid	
Dinitroaniline	Trifluralin	2,6-Dinitro-N,N-dipropyl-4-(trifluoromethyl)aniline	

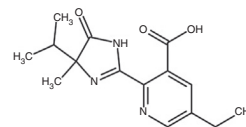
Pendimethalin

3,4-Dimethyl-2,6-dinitro-*N*-(pentan-3-yl)aniline

Imidazolinone

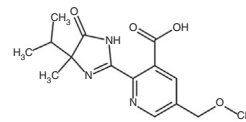
Imazethapyr

5-Ethyl-2-[4-methyl-5-oxo-4-(propan-2-yl)-4,5-dihydro-1H-imidazol-2-yl]pyridine-3-carboxylic acid



Imazamox

5-(Methoxymethyl)-2-[4-methyl-5-oxo-4-(propan-2-yl)-4,5-dihydro-1H-imidazol-2-yl]pyridine-3-carboxylic acid



of secondary metabolites essential for environmental adaptation (Tzin et al., 2012). Direct effects on microorganisms therefore may occur via this mode of action. Indeed, glyphosate has been shown to inhibit microbial growth in pure cultures at concentrations of 0.075 g/L (Shehata et al., 2013); however to our knowledge, thorough mechanistic studies have not been conducted to determine the exact mode or prevalence of toxicity in different microbial species. Unlike plants, many microorganisms are able to tolerate or overcome the toxic effects of glyphosate (eg, Drouin et al., 2010), presumably through upregulated EPSPS production, modified EPSPS structures or rapid metabolism/detoxification of the glyphosate molecule.

2.2 Chloroacetamides

Chloroacetamide herbicides, such as metolachlor and acetochlor, are commonly used herbicides. These are relatively mobile and persistent herbicides, especially metolachlor (Table 1). The mode of action of these herbicides is via inhibition of cell division and elongation in plants due to interference with a number of enzymes. The fatty acid elongase enzymes required for the synthesis of very long-chain fatty acids (VLCFAs) and geranylgeranyl pyrophosphate (GGPP) cyclization enzymes for gibberellin production are two particularly sensitive targets. Elongases are present in bacteria, fungi, and prokaryote cells, but they are a diverse family of enzymes with many different substrates and functions. We are not aware of any studies that have directly examined the effects of chloroacetamide herbicides on these enzyme systems in soil microorganisms or soil fauna. However, Bonnet et al. (2007) showed that nonspecific esterase activity was a more sensitive endpoint indicator of alachlor toxicity using a model bacterium and protist, rather than population dynamics; whereas the reverse was true for the herbicides diuron and glyphosate. This suggests that specific analysis of elongase and/or GGPP enzyme activity in soil may be appropriate endpoint assays for detecting effects of chloroacetamide herbicides. There is clearly more targeted mechanistic work that needs to be conducted in this area before any sound conclusions can be drawn.

2.3 Sulfonylureas and Imidazolinones

Sulfonylurea and imidazolinone herbicides are commonly used for cereal production and are effective at very low application rates compared to other

herbicides. Some of the herbicides from these classes (eg, chlorsulfuron) are relatively persistent and mobile (Table 1). Both herbicide classes act by inhibiting the enzyme acetolactase synthase (ALS), also known as acetohydroxyacid synthase (AHAS), which is responsible for the production of the branched chain amino acids leucine, isoleucine, and valine. The ALS enzyme is present in both plants and microorganisms and inhibition of microbial growth upon exposure to sulfonyleureas/imidazolinones is expected. Boldt and Jacobsen (1998) confirmed a toxic effect of sulfonyleurea herbicides on fluorescent pseudomonads isolated from an agricultural soil. At a concentration of 5 mg/L, sulfonyleurea herbicides reduced the growth rate of up to 20% of the strains tested. However, growth inhibition was relieved when branched chain amino acids were added to the culture media, implying that the mode of toxicity was an inhibition of ALS as hypothesized. Similar results were observed by Nelson and Duxbury (2008) in a study of 27 diverse soil bacterial isolates, with the authors concluding that the majority of soil microorganisms contain only one functional ALS enzyme that is sensitive to sulfonyleurea herbicides (Fig. 2).

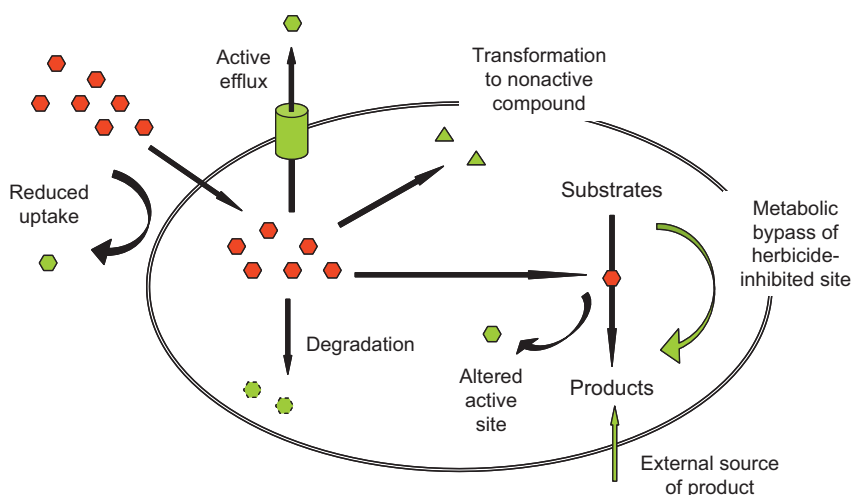


Figure 2 Resistance mechanisms by which microorganisms may avoid or overcome herbicide toxicities caused by enzyme inhibition (as represented by the conversion of substrates to products). Dark gray [red in online version] hexagons indicate active herbicide, while light-gray [green in online version] shapes represent inactive herbicides, herbicide metabolites or bypass systems.

2.4 Triazines, Phenylureas, Amides

Triazine, phenylurea, and (phenyl)amide herbicides block the quinone-binding site in photosystem II (PSII), leading to increased production of reactive oxygen species and subsequent damage to membranes, proteins, and DNA. The buildup of irreparable damage eventually results in plant death. Since the PSII site is specific to photosynthetic organisms, direct toxicity to (nonphotosynthetic) bacteria and fungi via this mode of action is unlikely. This does not rule out the possibility of indirect toxicities through other quinone-binding sites or unknown reactions, but suggests that potential effects of this kind may occur at higher concentrations than those toxic to plants and algae. These herbicide classes tend to be mobile and persistent and have been noted for their off-site migration potential (Table 1).

2.5 Phenoxy-carboxylic Acids

Phenoxy-carboxylic acids mimic the structure of the auxin class of plant hormones. Auxins regulate cell growth and division and thereby exert control over the shape and form of plants. They are especially important for plant nutrition as they initiate root formation and branching. There is also evidence for the role of auxins in mediating beneficial plant–microbial associations (Van Zwieten et al., 1995). When used as herbicides, phenoxy-carboxylic acids disrupt the hormone balance, causing growth abnormalities and injuries such as leaf curling, tissue swelling, and root splitting. Aside from plant-mediated effects, the mechanisms by which phenoxy-carboxylic acids may impact soil-dwelling organisms are unknown. High concentrations of phenoxy-carboxylic acids may have direct toxic effects, while it is plausible that low levels may interfere with plant–microbial signaling and act to alter the structure and function of microbial communities, in particular the balance between beneficial and pathogenic organisms. To our knowledge such a hypothesis has not yet been tested.

2.6 Dinitroanilines

Dinitroaniline herbicides such as trifluralin and pendimethalin halt cell mitosis (division) in plants by preventing the polymerization of tubulin to form microtubules (Morejohn et al., 1987). This effect dramatically inhibits the growth of plant tissue, particularly roots, causing seedling death. Although it was previously thought that tubulins were only present in eukaryotes, evidence suggests that the prokaryotic cell-division protein FtsZ is a structural

homolog (Amos and Lowe, 1998). Nevertheless, the dinitroaniline herbicides only appear to affect tubulins found in plants, algae, and protozoa, but not animals, fungi (reviewed in Morrisette and Sept, 2008) or, as far as we are aware, prokaryotes. The effects on protozoa may shift the ecological balance of the soil and have a consequent impact on beneficial processes, but to our knowledge no such effects have been mechanistically studied. Both trifluralin and pendimethalin have a high binding affinity to soil and are persistent in nature, with half-lives of 180 and 90 days respectively (Table 1). Trifluralin shows strong affinity (binding) for soil and is susceptible to rapid volatilization losses after application and therefore often needs to be incorporated in soil.



3. SOIL BIOLOGY: COMMUNITY STRUCTURE, FUNCTION, AND ASSESSMENT

Living organisms play a critical role in the distribution, transformation, availability and sequestration of carbon, nutrients and toxicants in soil, and therefore crop production. One of the current challenges in soil science is defining which organisms contribute to, or influence, specific functions and how biological communities adapt to environmental changes without losing the ability to support plant growth and other agronomic goals. In the context of this review, we have taken an “agricentric” stance in that our discussion focuses on the processes relevant to sustainable crop production. Although some overlap no doubt exists with processes relevant to natural ecosystems, we have deliberately avoided discussion about herbicide impacts in these systems. We should also point out that although soil biology encompasses a wide range of organisms including plant and animals, our discussion here is limited to microbial and mesofaunal communities, as well as earthworms. Mesofauna include those organisms which are less than 2 mm in size, such as nematodes, collembola, and mites. Earthworms have been included in this discussion because of their well-established role in soil fertility and common use as a bioindicator in soil toxicity studies (Paoletti, 1999).

3.1 Biological Communities and Functions Relevant to Crop Production

One of the main services provided by soil organisms is the turnover of organic matter. This process involves multiple scales of breakdown and

transformation, from the macroscopic cutting and breakdown of particulate organic material, to the enzymatic cleavage of polymers into monomers as occurs in the hydrolysis of cellulose to glucose. Organic matter contributes strongly to the available water content of soils (Hudson, 1994), but the benefits to water availability appear to improve over time as the organic matter ages in soil (De Silva and Cook, 2003). Organic matter turnover also liberates nutrients for crop growth, while the balance between turnover and stabilization of organic matter determines the loss of C from the system as dissolved organic material or gaseous molecules, mainly CO₂ and CH₄ (Baldock and Nelson, 2000). As a consequence, organic matter turnover also plays a critical role in climate regulation.

Aside from organic matter turnover, soil organisms regulate nutrient availability through additional transformations of mineral and organic nitrogen (N), phosphorus (P), and other elements. The N cycle is of particular importance since N is a key requisite for high crop yields and grain/fodder quality, and is strongly influenced by biological processes. Nitrogen fixation, that is the conversion of atmospheric N₂ to organic N, is carried by both free-living bacteria and also symbiotic plant–microbial associations. Symbiotic N-fixation in legume crops is especially important as contributions of N from fixation can be in the order of 100 kg N per hectare per crop (Peoples and Craswell, 1992). In terms of ecosystem services, the value of N-fixation can be readily quantified as it directly substitutes for chemical N fertilizer input. After organic (reduced) N is introduced into the soil, either through biological N-fixation or as urea fertilizer application, it is gradually mineralized to ammonium. Under aerobic conditions, ammonium will be oxidized mainly to nitrite, nitrate, and smaller amounts of gases (NO, NO₂, and N₂O) in the process of nitrification. Due to the predisposition of nitrate to leaching and also denitrification to N₂O, biological processes of ammonification, nitrification, and denitrification in soil strongly regulate the availability of N to agricultural crops.

The soil biology also influences the availability of P and other elements to crops. Of special importance is the symbiotic association of plants with mycorrhizal fungi. These associations mutually benefit both partners, through a flow of reduced carbon substrates from the plant to the fungi in return for other nutrients, especially P and microelements (Smith and Read, 1997). Other rhizosphere microorganisms contribute to plant nutrition through the production of organic acids that can release mineral-bound phosphates and siderophores that chelate micronutrients in the soil solution

(Vessey, 2003). Members of the genus *Bulkholderia*, *Enterobacter*, and *Pseudomonas* are particularly well represented in this group of rhizobacteria (da Costa et al., 2014; Khan et al., 2009).

The abilities of certain soil organisms to cause plant disease, and for some soils to suppress disease, are also important processes with direct relevance to sustainable crop production. Organisms which can cause direct crop damage include insects, nematodes, fungi, and bacteria. The taxonomy of insect pests and plant-parasitic nematodes is well known and commercial diagnostic services are available for identifying and enumerating these species in soil samples. Similarly, the most prevalent disease-causing fungi and bacteria are also well characterized, such that commercial testing for the major diseases in Australian cropping systems is available (Ophel-Keller et al., 2008). Less well known are the mechanisms by which some soils suppress the occurrence of disease, despite the presence of disease-causing organisms—but this is mainly because of the diversity of mechanisms and complexity of interactions involved (Mazzola, 2002). Such complexity means that the role of particular microbial groups or individual species in inhibiting plant pathogens is also challenging to decipher (Mazzola, 2002).

3.2 Methods for Assessing Community Structure

One of the most difficult aspects in assessing the impact of herbicides on soil biology and their functions is appraising which methods are the most appropriate for use and how different methods relate to each other. The complexity of this task has increased dramatically in the last few decades with the rapid development of molecular methods for monitoring community structure and function. We give a brief overview here of the methods that feature throughout this article.

In terms of assessing the community structure of mesofauna and earthworms, microscopic observation is routinely used. Although well-established, microscopy is relatively time-consuming and requires some specialist skills in identification and taxonomy (Ritz and Trudgill, 1999). Additional factors to consider include the size of the sample needed and which method of extraction will give unbiased and maximum recoveries (Neher et al., 1995). More recent molecular methods have been developed in an attempt to decrease sample processing time and cost (Chen et al., 2010; Griffiths et al., 2006). Although some evidence of bias has been detected (Donn et al., 2011), molecular methods are capable of rapidly and accurately

differentiating nematode communities responding to changes in the soil environment (Donn et al., 2012).

Soil microbial community structure can be assessed through a number of different means, including culture-dependent physiological characterization and culture-independent techniques based on nucleic acids and fatty acid profiles. Culture-dependent techniques involve growing and isolating or identifying discrete pure colony-forming units for enumeration and characterization (Hill et al., 2000). Such techniques are relatively inexpensive, quick, and easily performed without specialized equipment. An additional benefit is that colonies of interest can be mass cultured to further explore the mechanisms behind their functioning and interactions with other organisms. Groups of interest (eg, phosphate solubilizers, pathogens, siderophore producers) may also be enumerated through culturing on selective media. Microbial community characterization as a whole, without isolation of individual colonies, can also be achieved through culture-dependent techniques, commonly known as community-level physiological profiling (CLPP) (Hill et al., 2000). Two of the more common, high throughput formats include the Biolog and Microresp system. The Biolog system involves inoculation of artificial media containing a specific growth substrate with diluted soil slurry and measuring the color change in the media to determine growth (Garland and Mills, 1991). In contrast, the Microresp system involves dosing a soil microcosm with a growth substrate and using an alkaline gel to capture CO₂ respired from the soil (Campbell et al., 2003). The CO₂ is quantified colorimetrically through changes to a pH indicator in the alkaline gel.

The primary drawback of culture-dependent methods is that results can be biased toward those community members who are dominant, fast growing, and/or nonfastidious (Hill et al., 2000). Changes may also occur in the community structure and function during the cultivation period. Information gleaned through these methods is limited to a small subset of the microbial population and does not truly represent the entire community or the ecological dynamic interplay. By comparison, culture-independent molecular methods are generally more inclusive and are a powerful means to describe microbial diversity in ecosystem maintenance. However, it must be noted that some molecular methods may introduce different biases—for example, through different extraction methods or primer choice in PCR—and these should be considered when interpreting results (Hirsch et al., 2010). A number of reviews are available on the subject of culture-independent methods for microbial community and functional analysis and the reader is

directed to these for a more in-depth explanation of the techniques (Hirsch et al., 2010; Rincon-Florez et al., 2013). A summary of the advantages and disadvantages of these methods is given in Table 2).

3.3 Methods for Assessing Soil Biological Community Function

Assessing changes to soil biological community function can be achieved via macroscopic observations, measurement of chemical pools and fluxes, and microbial culture-dependent and culture-independent approaches. Many of these are advocated for use in defining endpoint toxicities in “terrestrial model ecosystems” as described by Weyers et al. (2004). Macroscopic observations encompass a broad range of assessments including plant root and shoot growth or form, disease symptoms, degradation of organic biomass (eg, calico strips), and feeding activity (eg, bait lamina). These measures are useful for longer term monitoring at higher tier scales. Similarly, measurement of pools and fluxes of chemical species, such as soil nitrate, ammonium, and phosphate concentrations or CO₂ and N₂O emissions, over time can give valuable insight into changes in soil biological community function. Such measures are routinely reported in studies assessing the impact of herbicides on soil biology.

More detailed mechanistic information can be gained by applying culture-dependent and independent methods to examine specific microbial functions. Results from CLPP assays (described in Section 3.2) can provide direct information about the ability of a microbial community to metabolize a specific organic compound. Another popular approach is to measure the activities of a diverse set of enzymes acting on different chemical pathways involved in C, N, P, and S cycling (Caldwell, 2005). The recent development of fluorescent substrates and method formatting in microwell plates (Marx et al., 2001) has increased the sensitivity and speed of many enzyme assays, allowing for greater precision and sample throughput. However, as with culture-dependent characterization of microbial community structure, the use of high substrate levels can induce a level of bias toward fast growing and dominant members of the community. Additional considerations also need to be given to the environmental conditions under which assays are performed, particularly temperature and pH, as variations in these parameters can strongly influence enzyme activities (German et al., 2011; Niemi and Vepsäläinen, 2005).

Use of culture-independent, nucleic acid-based methods for functional characterization of soil microbial communities is becoming more frequent

Table 2 Culture-independent methods for assessing soil microbial community biomass and diversity.

Endpoint	Method	Advantages	Disadvantages
Biomass	CFE	<ul style="list-style-type: none">• Measurements of microbial biomass can be done in recently added and freshly decomposed substrates	<ul style="list-style-type: none">• Clay soils may need to be corrected for the amount of chloroform C added to assess the concentration of biomass C
	PLFA	<ul style="list-style-type: none">• Sensitive detection and accurate quantification of different microbial groups• Rapid and efficient• Useful information on the dynamics of viable bacteria	<ul style="list-style-type: none">• Time consuming• Low number of samples can be treated at the same time
	Q-PCR	<ul style="list-style-type: none">• Reproducible• Quick, accurate and highly sensitive method for sequence quantification that can also be used to quantify microbial groups• Relatively cheap and easy to implement• Specific amplification can be confirmed by melting curve analysis.	<ul style="list-style-type: none">• Can only be used for targeting of known sequences.• DNA impurities and artefacts may create false-positives or inhibit amplification.

Community structure and diversity	DGGE/TGGE	<ul style="list-style-type: none"> • Sensitive to variation in DNA sequences • Bands can be excised, cloned and sequenced for identification 	<ul style="list-style-type: none"> • Time consuming • Multiple bands for a single species can be generated due to micro-heterogeneity • Can be used only for short fragments • Requires optimisation to obtain good separation of DNA from complex communities • Limited to dominant community members
	SSCP	<ul style="list-style-type: none"> • Community members can be identified • Screening of potential variations in sequences • Helps to identify new mutations 	<ul style="list-style-type: none"> • Short fragments • Lack of reproducibility • Several factors like mutation and size of fragments can affect the sensitivity of the method
	T-RFLP	<ul style="list-style-type: none"> • Enables analyses of a wide array of microbes • Highly reproducible 	<ul style="list-style-type: none"> • Artefacts might appear as false peaks • Distinct sequences sharing a restriction site will result in one peak. • Unable to retrieve sequences
	RISA/ARISA	<ul style="list-style-type: none"> • High resolution when detecting microbial diversity • Quick and sensitive 	<ul style="list-style-type: none"> • More than one peak could be generated for a single organisms • Similar spacer length in unrelated organisms may lead to underestimations of community diversity
	LH-PCR	<ul style="list-style-type: none"> • Results are reproducible • Easy and rapid • Efficient and reliable 	<ul style="list-style-type: none"> • Limited by the bacterial species known in public databases • Not enough information is available for fragment length on databases to compare LH-PCR lengths with environmental microorganisms.

(Continued)

Table 2 Culture-independent methods for assessing soil microbial community biomass and diversity.—cont'd.

Endpoint	Method	Advantages	Disadvantages
	RAPD	<ul style="list-style-type: none"> • Suitable for unknown genomes • Requires low quantities of DNA. • Efficient, fast and low cost 	<ul style="list-style-type: none"> • Low reproducibility • Sensitive to reaction conditions
	ANDRA	<ul style="list-style-type: none"> • Highly useful for detection of structural changes in simple microbial communities • No special equipment required 	<ul style="list-style-type: none"> • More applicable to environments with low complexity • Several restrictions are needed for adequate resolution • Labour- and time-intensive • Different bands can belong to the same group
	FISH	<ul style="list-style-type: none"> • Allows detection and spatial distribution of more than one samples at the same time 	<ul style="list-style-type: none"> • Autofluorescence of microorganisms • Accuracy and reliability is highly dependent on specificity of probe(s)
	DNA ARRAY	<ul style="list-style-type: none"> • Analyzes a vast amount of genetic information simultaneously • Requires the construction of an array and access to a scanner 	<ul style="list-style-type: none"> • Issues with specificity/cross hybridization • Requires normalization • Sensitivity and reproducibility can be problematic • Limited to known gene sequence probes and presence on the array

Next Generation Sequencing (16S rRNA amplicon sequencing)	<ul style="list-style-type: none"> • Rapid method to assess biodiversity and abundance of many species/organizational taxonomic units simultaneously and at a considerable depth compared to the methods that have been available so far 	<ul style="list-style-type: none"> • Relatively expensive • Replication and statistical analysis are essential • Computational intensive • Challenging in terms of data analysis
Next Generation Sequencing (meta- genomics)	<ul style="list-style-type: none"> • Biodiversity can be studied in more detail • Captures polymorphism in microbial communities • Reveals the presence of thousands of microbial genomes simultaneously • Provides information about the functions of microbial communities in a given environment 	<ul style="list-style-type: none"> • High cost • Data analysis is challenging and time consuming • Difficult to use for low-abundance communities. • High biodiversity in soil leads to many incomplete genomes • Current sequencing methods and computing power still in its infancy relative to the high biodiversity in soil

Methods include CFE, chloroform fumigation-extraction; PLFA, phospholipid fatty acid analysis; Q-PCR, quantitative PCR; DGGE, denaturing gradient gel electrophoresis; TGGE, temperature gradient gel electrophoresis; SSCP, single-strand conformation polymorphism; T-RFLP, terminal restriction fragment length polymorphism fingerprinting; ARISA/RISA, automated ribosomal intergenic spacer analysis; LH-PCR, length-heterogeneity PCR; RAPD, random amplified polymorphic DNA; ARDRA, amplified ribosomal DNA restriction analysis; FISH, fluorescence in situ hybridization.

Source: Adapted from [Rincon-Florez](#)

(Rincon-Florez et al., 2013). Methods include quantitative polymerase chain reaction (q-PCR) of functional genes, hybridization of functional genes in an array format, and sequencing extracts of soil mRNA (Table 3). Functional gene arrays and metatranscriptomics can give a very detailed snapshot of microbial community activity at a particular point in time, but high cost and relatively complex data processing currently limits the widespread application of these technologies. Furthermore, all three methods remain partially limited by the knowledge gaps in relating specific nucleic acid sequences to a known function.



4. EFFECTS ON SOIL BIOTA AND COMMUNITY STRUCTURE

4.1 Microbial Communities

4.1.1 *Glycine: Glyphosate*

Numerous studies have found that glyphosate applied at standard application rates (PEC = 3 mg/kg, Table 1) has little impact on the microbial biomass in soil, and stimulation rather than inhibition is more commonly observed (Table 4). In some cases, glyphosate has a variable effect over time, with increases, decreases, or no effects being observed periodically after the initial exposure (Abdel-Mallek et al., 1994). These findings for soil application are interesting, since glyphosate at 5 mg/L (representing a maximum concentration in soil solution soon after application) applied in vitro reduced growth of 21 out of 22 fungal species isolated from a boreal forest soil when challenged with increasing doses of formulation (Round-up) (Tanney and Hutchison, 2010). This highlights the large differences between laboratory-based dose-response studies in vitro, compared with studies in soil where sorption and other factors likely reduce acute toxicities.

Even though the effect of glyphosate on the total microbial biomass appears to be negligible, this does not rule out an effect on finer-scale population dynamics. A number of studies examining these dynamics using high-throughput and more advanced culture-independent methods are discussed later in the chapter.

The addition of the recommended field-rate concentration of glyphosate (5 kg/ha corresponding to 50 mg/kg) to two different forest soils caused no major changes in microbial community structure assessed by CLPP, PLFA, and standard cultural and microscope methods (Ratcliff et al., 2006). A

Table 3 Methods for assessing soil microbial community function.

Endpoint	Method	Advantages	Disadvantages
FUNCTION	Enzyme activity assays (FDA, DHA)	<ul style="list-style-type: none"> • Low-cost, easy and fast method to measure microbial activity for soil samples • High sensitivity to changes in the soil environment 	<ul style="list-style-type: none"> • Enzyme activities can be contaminated by external sources, e.g. plant matter • Limited substrate availability with a bias towards hydrolytic enzymes • Can only be used for targeting of known sequences. • DNA impurities and artefacts may create false-positives or inhibit amplification. • Requires the construction of an array and access to a scanner • Issues with specificity/cross hybridization • Requires normalization • Insufficient sensitivity and reproducibility can be problematic • Limited by the presence of probes on the array • Issues with RNA extraction from soil
	qPCR	<ul style="list-style-type: none"> • Quick, accurate and highly sensitive method for quantification of functional genes • Relatively cheap and easy to implement 	
	Functional Gene Arrays (RNA-based)	<ul style="list-style-type: none"> • Analyzes a vast amount of genetic information simultaneously 	

(Continued)

Table 3 Methods for assessing soil microbial community function.—cont'd.

Endpoint	Method	Advantages	Disadvantages
	Next Generation Sequencing (Metatranscriptomics)	<ul style="list-style-type: none"> • Allows rRNA and/or mRNA profiling and quantification without prior knowledge of sequence • Provides a snapshot of microbial transcripts at the time of sampling that may allow deduction of microbial ecosystem function • Helps to understand the response of microbial communities to changes in their environment 	<ul style="list-style-type: none"> • Many issues with isolation of RNA from soil • mRNA isolation and often amplification are required for gene expression analyses • Current sequencing methods, data bases and computing power are not sufficient yet to cover the high biodiversity in soil.

Abbreviations: FDA, fluorescein diacetate; DNA, dehydrogenase; qPCR, quantitative polymerase chain reaction; and RNA, ribonucleic acid.

Source: Adapted from [Rincon-Florez](#)

Table 4 Summary of studies investigating the effect of glyphosate on soil microbial biomass.

Application rate (cf. average agricultural rate of 2.2 kg/ha)	Soil organic matter (%)	Soil pH	History of glyphosate application	Total microbial biomass	Bacteria	Fungi	Actinomycetes	References
>2.2 kg/ha	7	6.5	NR	NR	↑, until 30 d	No effect	No effect	Wardle and Parkinson (1990)
	3.5	7	NR	NR	↑, until 108 d	NR	NR	Sihtmäe et al. (2013)
	1.9	6.2	NR	No effect	NR	NR	NR	Liphadzi et al. (2005)
	2.79	6.9	Yes	↓, 4 d; No effect, 45 d	NR	NR	NR	Gomez et al. (2009)
< 2.2 kg/ha	7	6.5	NR	NR	No effect	No effect	No effect	Wardle and Parkinson (1990)
	3.5	7	NR	NR	↑, until 21 d	NR	NR	Sihtmäe et al. (2013)
	2.3	5.7	No	NR	No effect	No effect	↑, 32 d	Araújo et al. (2003)
	2.3	5.9	Yes	NR	↑, 32 d	No effect	No effect	Araújo et al. (2003)
	2.6	5.6	No	NR	No effect	No effect	No effect	Araújo et al. (2003)

(Continued)

Table 4 Summary of studies investigating the effect of glyphosate on soil microbial biomass.—cont'd.

Application rate (cf. average agricultural rate of 2.2 kg/ha)	Soil organic matter (%)	Soil pH	History of glyphosate application	Total microbial biomass	Bacteria	Fungi	Actinomycetes	References
	2	5.2	Yes	NR	No effect	No effect	No effect	Araújo et al. (2003)
	1.9	6.2	NR	No effect	NR	NR	NR	Liphadzi et al. (2005)
	2.79	6.9	Yes	No effect, 4 d; ↑, 45 d	NR	NR	NR	Gomez et al. (2009)
	2.4	3.8	NR	No effect	No effect	No effect	No effect	Stratton and Stewart (1992)
	35.6	5.2	NR	No effect	NR	NR	NR	Houston et al. (1998)
	3.4	4.8	NR	No effect	NR	NR	NR	Houston et al. (1998)
	NR	NR	NR	NR	↓, 180 d	↓, 60 d	NR	Chakravarty and Chatarpaul (1990)

NR, not reported. Arrows indicate a statistically significant ($P < 0.05$) increase (↑) or a decrease (↓) in biomass relative to control (no herbicide) treatments at a specific time point (d, days) after application. Treatments causing an effect of extended duration are reported as having an effect until a specific time point after application.

higher rate of 100-times the field-rate concentration (ie, 500 kg/ha), reflecting an undiluted chemical spill, produced a significant enrichment of bacteria and minimal change to the fungal community (Ratcliff et al., 2006). Similarly, glyphosate (2.5 kg/ha) had no effect on microbial diversity, as measured by ester-linked FAMEs, 14 days after application in three different seasons in soybean rhizosphere or bulk soil. Laboratory incubations showed slight alteration in community structure (FAME) 3 days after relatively high application rates of 47 or 150 mg/kg, but these values reconverged at 7 days (Weaver et al., 2007). Glyphosate application as Roundup PowerMAX temporarily lowered the total microbial biomass in the rhizosphere of glyphosate-resistant soybean grown in soil that had no previous exposure to glyphosate, but caused no changes in the microbial community structure as measured by ester-linked FAME (Lane et al., 2012). Repeated applications of glyphosate as Roundup PowerMAX (6 applications of 118 mg/kg over 6 months, equivalent to 88.5 kg/ha per application) also did not significantly change the microbial community structure as measured by ester-linked FAME (Lane et al., 2012) and a field survey could not detect any significant effect of glyphosate on total microbial biomass or fungal and bacterial biomass and ratios using PLFA (Rosenbaum et al., 2014). Single and repeat applications of glyphosate as Roundup WeatherMAX (49 mg/kg) had only a minor effect on FAME profiles, but clone libraries and pyrosequencing showed increases in proteobacteria, specifically Burkholderiales (Lancaster et al., 2010).

Using CLPP and DNA fingerprinting, Zabaloy et al. (2012) found little to no effect of standard glyphosate rates on the microbial community structure by either C-substrate utilization or T-RFLP. Similarly, glyphosate at both conventional application rates and 10-times higher had little impact on the T-RFLP profiles of bacterial communities and total DNA extracted from three different soils (Zabaloy et al., 2012). Application of 1.8 kg/ha of glyphosate to Roundup Ready corn also had no effect on the structure of the rhizosphere fungal community as measured using T-RFLP (Hart et al., 2009). Interestingly, glyphosate (50 and 500 mg/kg) applied as Roundup increased the CLPP diversity in both triticale and mixed triticale-pea rhizospheres, 15 days after application, even though diversity change could not be detected by DGGE (Mijangos et al., 2009). At the same time, the ability of the microbial community to metabolize the surfactant Tween 20 was increased, suggesting a strong influence by the formulation constituents on community-level physiological profiles. However, 30 days after application, the differences were inconsistent between application rates and plant community

rhizospheres, suggesting potential effects are short-lived and difficult to generalize (Mijangos et al., 2009). This supports the findings of Lupwayi and Blackshaw (2012) that periodic increases and decreases in the CLPP bacterial diversity occurred in corn rhizospheres and bulk soil after annual glyphosate applications over a 5-year monitoring period.

More recent attempts to delve into community structural changes have utilized rapidly-developing next-generation sequencing techniques, which have greater sensitivity in detecting changes to nonculturable and underrepresented microbial taxa. The findings from one of the first studies to use next-generation sequencing supported earlier work using different methods by showing that repeated application of glyphosate at a standard rate (0.72 kg/ha) to maize grown on two soils had insignificant effects on microbial community structure (Barriuso et al., 2011a). However, studies by the same authors also detected transiently altered microbial community composition in response to glyphosate application, but these changes were small compared to the herbicide mixture "GTZ" (containing 2.2 kg/ha aceto-chlor and 0.87 kg/ha terbuthylazine), which had a more persistent effect on reshaping the microbial community (Barriuso et al., 2010; Barriuso and Mellado, 2012). Pyrosequencing of culturable bacteria also showed a reduction in diversity caused by glyphosate, but even greater reduction caused by GTZ (Barriuso et al., 2011b). Taken as a whole, these results suggest that the application of glyphosate at or near recommended field rates has no demonstrable consistent, significant impact on soil microbial community structure.

A herbicide that has similar chemistry as glyphosate, that is, glufosinate, appears to have a greater impact on microbial communities. Low levels of glufosinate-ammonium (1, 10 mg/kg) increased the culturable counts of cellulose degraders and ammonia-oxidizing bacteria, but dramatically reduced the actinomycete counts and these did not recover even by the end of the experiment, 40 days after application (Pampulha et al., 2007). Glufosinate application (3 kg/ha) caused transient changes in the eubacterial and *Pseudomonas* population structure as detected by PCR-DGGE (Gyamfi et al., 2002) and also altered the active bacterial communities in canola rhizosphere (16S rRNA DGGE), with generally higher active populations of key groups (Sessitsch et al., 2005). However, Schmalenberger and Tebbe (2003) did not detect changes in the bacterial community structure after glufosinate application to conventional or transgenic herbicide-tolerant maize, using molecular methods. Likewise, Ernst et al. (2008) found no impact on bacterial community structure when glufosinate (0.6 kg/ha) was applied to glufosinate-resistant rape/maize. The contradictory nature

of these results may be a consequence of different monitoring periods and environmental conditions, as [Griffiths et al. \(2008\)](#) found altered microbial diversity (CLPP and ester-linked FAME) at 6 weeks after herbicide application but no differences at 12 weeks.

4.1.2 Chloroacetanilides

Most studies examining the impact of chloroacetanilide herbicides on microbial community size and structure have focused on the chemical butachlor. Generally speaking, butachlor application at standard rates (<10 mg/kg) was found to have little effect on the size of the soil microbial community under laboratory ([Xia et al., 2011](#)) or field conditions ([Singh and Ghoshal, 2010](#)). At these rates, effects on microbial community structure are also insignificant as determined by random amplified polymorphic DNA (RAPD) analysis ([Wang et al., 2007, 2009](#)), but they may have a temporary effect on functional diversity as measured by CLPP ([Fang et al., 2009](#)). Butachlor application rates higher than 10 mg/kg have been reported to cause a more marked and long-lasting reduction of soil bacterial diversity ([Wang et al., 2007, 2009](#)). Similarly, application of alachlor and metolachlor at rates of 10 mg/kg did not have any pronounced effect on bacterial and fungal populations ([Dzantor and Felsot, 1991](#)). In contrast, a relatively low application rate (0.85 mg/kg) of another chloroacetanilide herbicide, acetochlor, stimulated bacterial populations, while fungal growth exhibited a reverse trend ([Bai et al., 2013](#)). The microbial composition as measured by PLFA was significantly altered by acetochlor in the early stage (15 days) after application; thereafter (19–35 days), any impacts on soil microbial communities were attenuated and eventually undetectable ([Bai et al., 2013](#)). Another study examining the effect of high concentrations of acetochlor (50–250 mg/kg, relative to a PEC of about 8 mg/kg) on soil fungal communities by DGGE found that acetochlor had a transitory effect on fungal diversity, returning to background levels by 60 days ([Xin-Yu et al., 2010](#)). Nevertheless, the actual community structure had shifted at 60 days from controls in all acetochlor treatments ([Xin-Yu et al., 2010](#)). Applications of metazachlor (0.5 kg/ha) also altered the PLFA community structure in the rhizosphere of nontransgenic canola ([Ernst et al., 2008](#)), but measurements were made at an unspecified time, so no assessment of resilience could be made. Pretilachlor (0.45 kg/ha) had no significant impact on MBC or PLFA profiles in a rice paddy soil ([Murata et al., 2004](#)). As with glyphosate there was little effect of chloroacetanilide herbicides on microbial community structure and where effects were present they were not typically consistent among studies, making it difficult to draw conclusions.

4.1.3 *Sulfonylureas and Imidazolinones*

Research into the effect of sulfonylurea herbicides on microbial populations suggests that most compounds from this class applied at conventional rates have no impact on microbial biomass, while higher rates may temporarily reduce microbial biomass. For example, rimsulfuron at 0.025 kg/ha had no effects on microbial biomass C (MBC), but higher doses at 10 and 100 times the conventional rate reduced MBC (Perucci et al., 1999, 2000; Vischetti et al., 1997). The onset and magnitude of these effects were dependent on temperature and humidity; however, they were generally slight (<20%) and transitory. As with rimsulfuron, chlorsulfuron applied at a conventional rate (0.01 mg/kg) also had no significant effect on MBC or microbial biomass nitrogen (MBN), while 10 and 100 times higher doses significantly reduced MBC and MBN by around 25 and 50%, respectively (El-Ghamry et al., 2000). But unlike rimsulfuron, higher application rates resulted in sustained suppression of microbial biomass even after 45 days (El-Ghamry et al., 2000). A low-rate sulfonylurea mixture (0.01 mg/kg metsulfuron + 0.01 mg/kg bensulfuron) significantly reduced MBN and MBC in the first 10 days following application, after which they recovered. Higher rates had an even greater (negative) impact on MBN and MBC (El-Ghamry et al., 2001). Similar results were observed when bensulfuron-methyl was applied without metsulfuron at a conventional rate (0.01 mg/kg), wherein it transiently reduced MBC and MBN in the first week after application. Higher rates (10X; 100X conventional) also affected MBC and MBN and increased the time taken to return to control levels (El-Ghamry et al., 2002).

In terms of microbial community structure, bensulfuron-methyl at rates of 0.067 mg/kg and higher reduced the counts of culturable bacteria until 60 days, whereas the effects on fungi and actinomycetes were inconsistent and transient. Overall bacterial diversity measured by DGGE was only significantly affected at application rates of 0.355 mg/kg or higher in a soil with pH 7.2 (Lin et al., 2008). This differs from another study, which found no significant effect of bensulfuron-methyl on bacterial diversity measured by DGGE when applied at 0.051 or 0.51 mg/kg to a soil with pH 4.7 (Saeki and Toyota, 2004). Similarly metsulfuron-methyl (1 mg/kg) had no effect on CLPP diversity (Zabaloy et al., 2008). The discrepancy between these studies may be related to soil pH, as sulfonylurea herbicides are known to break-down much more rapidly at acidic pH (Sarmah and Sabadie, 2002). Long-term (5 or 10 year) application of chlorimuron-ethyl to soybean fields significantly reduced culturable bacteria and actinomycetes, but increased fungal counts. Molecular profiling of bacterial and fungal DNA also indicated

significant shifts in community structures of both microbial groups, corresponding to reduced diversity (Zhang et al., 2011).

Limited information is available about the effect of imidazolinone herbicides on soil microbial community structure. Imazamox (0.1 kg/ha) reduced MBC by 19–22%, but levels subsequently recovered (Vischetti et al., 2002). Similarly, imazethapyr added at a conventional rate (0.12 kg/ha, approximately equivalent to 0.16 mg/kg) had no effect on MBC (Zhang et al., 2010b) or a temporary inhibition (Xu et al., 2013), but levels recovered within 30 days after application. Higher rates of application caused greater negative impacts, but levels also quickly recovered in the same timeframe. Fluctuations in fungal and bacterial PLFAs and PLFA patterns were also observed at these rates, but they were inconsistent over time and did not persist beyond 60 days (Zhang et al., 2010b; Xu et al., 2013).

4.1.4 Triazines

An early study on the effect of the s-triazine herbicide atrazine found that application rates of 30 or 100 mg/kg to a loam soil resulted in increased populations of culturable actinomycetes, bacteria, and fungi over those in nontreated soil (Percich and Lockwood, 1978). The increases were in proportion to the quantity of atrazine applied, and effects persisted for at least 2 months. Application rates more consistent with label rates (10 mg/kg, slightly more than double our PEC of 4 mg/kg, Table 1) had no significant effect on culturable populations (Percich and Lockwood, 1978). Despite no effects on number of culturable organisms, application rates < 10 mg/kg still appear to alter the microbial community structure. In one study, atrazine applied at 5 mg/kg to five different soils induced a minor temporary (3 week) shift in microbial community as measured by PLFA (Mahía et al., 2011). Atrazine at 1, 2, and 3 mg/kg also caused a shift in the soil microbial community as monitored by DGGE: 10 days after application, some DNA bands showed increased intensity and up to 10 new bands could be detected, but by 30 days the differences were negligible (Briceño et al., 2010). A similar rate (1.5 mg/kg) of the related herbicide simazine also altered bacterial diversity in soil, by increasing the relative proportion of α - and β -proteobacteria and decreasing γ -proteobacteria up to 30 days after application (Girardi et al., 2013; Caracciolo et al., 2010). Prometryn-treated soils also showed some differences in molecular profiles (by 16S PCR-DGGE and amplified ribosomal DNA restriction analysis) at 100 mg/kg, but the differences usually involved detection of additional bands, suggesting enrichment of prometryn-degrading organisms (Crecchio et al., 2001). However,

another triazine herbicide, hexazinone did not reduce soil microbial population at 1, 2, and 8 kg/ha (Chakravarty and Chatarpaul, 1990).

4.1.5 Phenoxycarboxylic Acids

The effects of 2,4-D application on microbial community size and structure are inconsistent. Devi et al. (2008) observed that 2,4-D application at conventional rates (0.5, 1, 2, and 4 kg/ha) temporarily reduced culturable bacteria and increased culturable fungi; but in another study, DGGE analysis did not detect any community shifts in soil treated with 2,4-D at 10 mg/kg (Macur et al., 2007). Moreover, the same rate (10 mg/kg) of 2,4-D butyl ester had no significant impact on culturable microbes, but did cause a significant shift in the PLFA profile of the soil microbial community (Zhang et al., 2010a). The only consistent finding is that microbial growth-dependent methods detect higher numbers of 2,4-D degrading organisms at conventional application rates of 5–10 mg/kg (Zabaloy et al., 2010; Macur et al., 2007). Together, these results highlight the difficulties in comparing or aggregating data from studies that use different methods, and imply that the use of multiple methods for assessment purposes is prudent in order to cover methodological biases.

4.1.6 Phenylureas, Amides

Data on the effect of phenylurea and associated PS-II inhibiting herbicides (eg, propanil) on microbial biomass and structure is sparse. Linuron applied to soil at a conventional rate (4 mg/kg) did not affect the number of culturable bacteria, fungi, nitrifiers, denitrifiers, or N-fixers, but some transient (<28 days) effects were observed at higher rates (Cycoń and Piotrowska-Seget, 2007). Metoxuron at 5 mg/kg had no effect on bacterial or fungal numbers, but fungal propagules were temporarily lower at 50 mg/kg and severely curtailed at 500 mg/kg, whereas total counts of bacterial propagules were greatly increased at 500 mg/kg. Propanil at all rates (1–100 mg/kg) did not affect community structure as measured by PCR-DGGE and amplified ribosomal DNA restriction analysis (ARDRA) (Crecchio et al., 2001)

4.1.7 Dinitroanilines

Trifluralin (0.1–0.8 mg/kg) had no consistent effect on fungi, bacteria, or actinomycete populations in either bulk soil or in wheat rhizospheres, although periodic increases and decreases were observed with respect to controls not receiving herbicide (Olson et al., 1984). Field application of trifluralin (1.0 kg/ha) into wheat at two different locations showed no effects

on soil fungi, bacteria, actinomycete, denitrifying bacteria, and nitrifier populations (Olson et al., 1984).

4.1.8 Other Herbicide Groups

Other minor-use herbicides appear to have little effect when applied at conventional label rates. Mefenacet did not affect diversity (DGGE) at 0.133 mg/kg (Ye et al., 2006) and had no significant impact on PLFA profiles in a rice paddy soil when applied at 1.05 kg/ha (Murata et al., 2004). Mesotrione applied at a conventional rate (0.45 kg/ha) or 10× higher (4.5 kg/ha) did not significantly impact the DGGE bacterial community structure (Crouzet et al., 2010), nor did it alter the diversity or structure of soil cyanobacterial communities (Crouzet et al., 2013). Napropamide only significantly affected community structure (16S-DGGE, CLPP, and PLFA) at 10× the conventional rate, while impacts at conventional rate were insignificant or quickly returned to normal (Cycoń et al., 2013a,b). Dinoseb applied to soil at 20 mg/kg did not significantly affect MBC, but at 60 mg/kg it significantly reduced MBC and levels did not recover by the end of the incubation (25 days) (Lin and Brookes, 1999). It is noteworthy that 60 mg/kg is a very high rate of application. Fluazifop-butyl had no significant effect on total fungal propagule populations at a standard rate equivalent to 0.6 mg/kg, but at higher rates of 3 and 6 mg/kg, it caused temporary reduction in fungal populations until 1 and 2 weeks after application, respectively (Abdel-Mallek et al., 1996). No significant impacts on microbial biomass and diversity were observed by application of bromoxynil (3 applications each month) at 10 mg/kg (Baxter and Cummings, 2008). However, repeat applications of 50 mg/kg dramatically altered diversity as measured by PCR-DGGE (Baxter and Cummings, 2008). Furthermore, a herbicide mixture consisting mainly of bromoxynil significantly reduced culturable fungi, actinomycetes, ammonia-oxidizing bacteria, and cellulolytic bacteria at all levels tested (1, 10, 100 mg/kg). All except fungi returned to control levels after 40 days (Pampulha and Oliveira, 2006).

4.2 Mesofauna and Earthworms

Numerous invertebrate groups influence soil and ecosystem services that contribute to plant growth and environmental systems in general. Nematodes, mites, collembolans, and earthworms all play a strong role in redistributing and breaking down organic matter, recycling nutrients, regulating microbial communities, and interacting with plant roots. Since they can be assessed visually, in terms of type and quantity, the effects of soil

disturbances are often more clear-cut than similar studies of microbial communities. Nevertheless, their mobility and interactive roles at lower and higher ecological tiers means that the agronomic effects of changes to these communities is sometimes difficult to predict.

Liphadzi et al. (2005) investigated the direct effects of five glyphosate rates ranging from 0.56–4.48 kg/ha on soil nematode communities in a controlled growth-chamber experiment and found that total nematode density and densities of individual populations (herbivores, fungivores, microbivores, omnivores) were unaffected by all of the tested application rates. Similarly, application of glyphosate (0.9 kg/ha) had no significant impact on the number of collembola in a maize-turnip rotation up to 40 days after application (Lins et al., 2007). Indeed, in terms of overall faunal response, Reinecke et al. (2002) found that glyphosate applied at 1.1 kg/ha to a vineyard soil stimulated, rather than inhibited, bait-lamina feeding activity.

Contrasting effects of glyphosate on earthworms have been described in the literature, with differences arising from earthworm ecotypes and the nature of the study. Dalby et al. (1995) observed little effect of glyphosate on endogeic earthworms (topsoil feeders), while epigeic earthworms (surface litter feeding) *Eisenia fetida* lost approximately half their body mass after 28 days when exposed to 8 mg/kg glyphosate (Yasmin and D'Souza, 2007). In another study, the impact of glyphosate at 10, 100, 500, and 1000 mg/kg on the earthworm *E. fetida* was a gradual and significant reduction in mean weight (50%) at all test concentrations (Correia and Moreira, 2010). In the same study, 2,4-D at 500 and 1000 mg/kg caused 100% mortality, while after 14 days, 30–40% mortality levels were observed at 1, 10, and 100 mg/kg. Clearly these rates are very high and the experiments were designed to assess the thresholds of adverse effects. However, *E. fetida* exhibited strong avoidance behavior in field soil treated with glyphosate at 1.44 kg/ha (Casabé et al., 2007). Avoidance behavior was also demonstrated when *E. fetida* were exposed to a formulation containing 5% glyphosate by mass (as isopropylamine salt); however the exact concentration was unclear (Verrell and Van Buskirk, 2004). According to Zaller et al. (2014), glyphosate also has the potential to alter ecological interactions between earthworms, mycorrhizal fungi, and above-ground plants, leading to reduced mycorrhizal plant colonization and modified earthworm feeding behavior. Since there are still many unknowns about the effects of glyphosate on mesofauna, particularly in complex ecological systems beyond those found in many laboratory incubation studies, further research in this area is warranted.

With regards to triazine herbicides, the compound simazine was found to have no significant effects on nematode or food-web structure when applied at 2.68 kg/ha (Sánchez-Moreno and Ferris, 2007), and no effect on bait-lamina feeding activity when applied at 3 kg/ha (Reinecke et al., 2002). Atrazine applied at conventional rates had no significant impact on the number of collembola (Lins et al., 2007) or earthworms (Chelinho et al., 2010); but did begin to inhibit earthworm reproduction in one soil when it was applied at 20.7 mg/kg, which is equivalent to a conventional application rate distributed only in the top 1 cm of soil (Svendsen et al., 2008). Atrazine also elicited a dose-response relationship with avoidance behavior, in which over 50% of worms avoided soil concentrations of 38 mg/kg—approximately 10-times higher than the PEC of 4 mg/kg (Amorim et al., 2008). Atrazine toxicity toward *E. fetida* in soils varies, with lethal concentrations to 50% of the population (LC₅₀) ranging from 15 mg/kg (Frampton et al., 2006) through to 110 mg/kg (NRA, 1997). The enchytraeid worm *Enchytraeus albidus* was more sensitive to atrazine than previous studies with *E. fetida* having an LC₅₀ of 12 mg/kg with 50% impact on reproduction at 2 mg/kg (Novais et al., 2010). Terbutylazine had no toxic effects on soil animals tested (microbes, opiod mites, two gamasid mite species, enchytraeids, and nematodes) when applied to soil at rates of 1–53 kg/ha as the active ingredient (Salminen et al., 1996). When it was applied as herbicide preparation, acute toxic effects on enchytraeids were observed, but only at rates above 10 kg/ha.

Although some adverse effects of herbicides have been reported, the diversity of experimental systems in various published studies means that the magnitude and duration of any effects may not be predictable. For example, Amorim et al. (2008) pointed out that *E. albidus* avoidance (50% of the test individuals) to phenmedipham in an initial study (Amorim et al., 2005) occurred at a concentration of 51 mg/kg, whereas a later study using the same conditions indicated a higher sensitivity, with 50% population avoidance at 7 mg/kg (Amorim et al., 2008). The authors suggested that the sensitivity to herbicides may therefore vary even within subpopulations of the same species, since different “batches” of individuals were used. This is further complicated by the fact that soil characteristics strongly influence the variability in responses (Amorim et al., 2008). Indeed, Griffiths et al. (2008) found that glufosinate or terbutylazine applied at conventional application rates reduced protozoa and microarthropods (mites and collembola) in maize rhizospheres in one soil but not another.

Furthermore, even if adverse effects are observed, it is difficult to determine the exact cause of the effect. For example, although Hartley et al. (1996)

found that terbutylazine (4 kg/ha) on two different apple orchard soils reduced earthworm numbers, they concluded that the effect was related to a lower weed density which reduced the food source, rather than direct herbicide toxicity. [Pelosi et al. \(2013\)](#) also found that an increase in herbicide usage correlated with decreased numbers of three different earthworms, but they could not determine whether the effects were direct, or whether they were an indirect result of the decreased OM inputs caused by conventional versus organic farming practices. Finally, [Cheng et al. \(2008\)](#) found that multiple herbicide treatments in a long-term (15 year) field experiment did not significantly affect nematode communities under turfgrass compared with equivalent controls without herbicide treatments; whereas high N-fertilizer treatment did alter nematode community structure. This shows that the effects of other management practices or experimental treatments have the potential to be misattributed to the effects of herbicides per se.



5. EFFECTS ON SOIL FUNCTIONS

5.1 Microbial Activity and C-Cycling

As highlighted in Section 3, the addition of herbicides to the soil can have both positive and negative impacts upon different members of the soil microbial community. Depending on the balance of these impacts, which can be either direct or indirect, alterations to soil C-cycling may also evolve. Direct effects include the herbicides being toxic to the microbes, which can result in a reduction of microbial biomass, and thence soil heterotrophic respiration and activity of OM decomposing and nutrient-cycling microbes. In contrast, herbicide addition can directly benefit soil microbes by providing a resource to support their growth ([Panettieri et al., 2013](#)). Herbicide impact on plants can also indirectly impact upon microbes. For example, where plant growth is suppressed, the levels of labile C input into the rhizosphere are expected to decline, which can have important consequences on those microbes that utilize root exudates. Where plants die following herbicide application, the remaining plant debris provides a resource to support microbial growth and activity. Further, a reduction in plant cover may result in an increase or decrease in soil temperature and water content, both of which affect rates of microbial activity. While not explored here in detail, herbicide impacts on plant community composition may also affect soil microbial

diversity and activity via plant-mediated selection of distinct microbial communities.

Soil microbial activity, in the context of soil C-cycling, can be measured in many ways. The most common measures in the context of herbicide impacts on soil microbes are heterotrophic respiration, the activity of enzymes involved in soil C-cycling, and organic matter decomposition and mineralization. In the following section these factors are considered for different herbicide classes.

5.1.1 Glycine

Generally, glyphosate applied at conventional rates (0.5–5 kg/ha) does not significantly reduce respiration. More commonly, respiration is either unaffected (Pereira et al., 2008; Houston et al., 1998; Busse et al., 2001; Wardle and Parkinson, 1991; Zabaloy and Gómez, 2008) or is stimulated (Means et al., 2007; Araújo et al., 2003), especially at higher application rates (Wardle and Parkinson, 1990b; Lancaster et al., 2006; Eser et al., 2007). Interestingly, in one study glyphosate application to virgin soil stimulated respiration yet application to a soil with previous exposure had no effect on respiration (Lane et al., 2012), whereas another study found the reverse was true (Zabaloy et al., 2012). This highlights the need for further studies that directly compare soils with different herbicide application histories. In one of the few cases where glyphosate application (5 kg/ha) to field plots temporarily reduced microbial respiration, such a response only occurred in plots where weeds were present (Wardle and Parkinson, 1992). The authors suggested that the impact of glyphosate was therefore a plant-mediated response, rather than a direct impact of the herbicide on soil microorganisms (Wardle and Parkinson, 1992).

Literature reports on the impacts of glyphosate on other enzyme indicators related to soil C-cycling usually follow a similar pattern to respiration measurements: that is, no change (Wardle and Parkinson, 1991) or an increase (Means et al., 2007; Araújo et al., 2003; Wardle and Parkinson, 1990b; Zabaloy et al., 2008; Panettieri et al., 2013). Interestingly, Haney et al. (2002) showed that glyphosate significantly stimulated soil microbial activity in a dose-dependent manner as measured by C and N mineralization, but did not affect soil microbial biomass at any rates. Although it was not clear whether higher C mineralization resulted from the breakdown of the herbicide or the native soil organic matter (Haney et al., 2002), Panettieri et al. (2013) speculated that increased

activities of enzymes such as dehydrogenase and β -glucosidase are probably a result of glyphosate acting as a source of easily available C. As far as we know, such a hypothesis is yet to be confirmed.

In contrast to the previously mentioned studies, [Damin et al. \(2012\)](#) found that glyphosate application to a black oat cover crop slowed the breakdown of plant residues. These authors hypothesized that this resulted from a change in the C:N content of the residues during the glyphosate-induced plant senescence, rather than the herbicide-inhibiting decomposer organisms. [Abdel-Mallek et al. \(1994\)](#) also found that plant-applied glyphosate inhibited the breakdown of broad bean residues, but accelerated the decomposition of wheat biomass. This further emphasizes the complexity of interactions and that the impact of glyphosate on plant-residue breakdown may be regulated by litter quality.

5.1.2 Chloroacetanilides

As with glyphosate, chloroacetanilide herbicides appear to have few negative impacts on respiration and general measures of microbial activity. Conventional application rates of alachlor (10 mg/kg), metolachlor (10 mg/kg), and butachlor (2.5 kg/ha) did not affect soil dehydrogenase activity ([Dzantor and Felsot, 1991](#); [Subhani et al., 2002](#)), while four rates of butachlor (5, 10, 50, 100 mg/kg) increased dehydrogenase activity in a dose-dependent manner for 3 weeks ([Xia et al., 2011](#)). Both the biomass and activity (respiration) of soil microbes were also enhanced with the application of pretilachlor ([Kumar et al., 2012](#)). One exception is the chloroacetanilide allidochlor, which reduced dehydrogenase activity over a period of 3 weeks when applied to soil at a rate of 10 mg/kg.

However, in contrast to the microbial activity measures mentioned earlier, there is some evidence that chloroacetanilides can disrupt other processes involved in C-cycling. In one study, the application of alachlor and metolachlor (at standard and high rates) reduced the number of cellulolytic microorganisms (especially bacteria) and their ability to colonize the cellulosic substrate ([Sahid and Yap, 1994](#)). These herbicides delayed the decomposition process, which recovered when there was no residual activity in the soil, after 12 weeks of incubation ([Sahid and Yap, 1994](#)). In another study, butachlor application significantly reduced CH₄ production in an alluvial rice soil in a dose-dependent response (from 5–100 mg/kg), even at the lowest level of application ([Mohanty et al., 2004](#)). This is interesting since metolachlor applied at 4.7 kg/ha had no significant effects on CH₄ emissions in an aerobic shortgrass steppe soil ([Kinney et al., 2004](#)). These results

highlight the importance of measuring specific functional activities, as opposed to more general measures such as respiration or dehydrogenase activity; but also reveal the importance of compound- and site-specific interactions, which make generalization difficult even with a chemical class. In many cases other factors such as soil moisture or nutrient status will have a much stronger influence on measures of microbial activities than herbicides (Muñoz-Leoz et al., 2012).

5.1.3 Sulfonylureas

Most studies have found also that sulfonylurea herbicides applied at conventional rates have no impact on respiration or other activity measures, while higher rates often lead to transient inhibition or stimulation (Table 5). Nevertheless, there are some exceptions to these general trends. For example, while metsulfuron-methyl applied at a range of concentrations (0.01–10 mg/kg) to an *acidic* soil had little impact, a low-rate application of 0.01 mg/kg to an *alkaline* soil depressed CO₂ evolution (Zabaloy and Gómez, 2008). Similarly, Sofo et al. (2012) also observed minor reductions in cumulative respiration from an alkaline soil, 30 days after the application of prosulfuron and triasulfuron at conventional rates. It is noteworthy that sulfonylurea herbicides being acidic are more mobile and bioavailable in neutral to alkaline conditions (Sarmah et al., 2000).

The impact of sulfonylurea herbicides on methane oxidation and cellulose decomposition activity have also been studied. The application of a mixture of nicosulfuron, atrazine, and dimethenamide did not significantly alter the soil methane oxidation rate or the abundance of methane oxidizers in another study (Seghers et al., 2005). However, as reported earlier for responses to glyphosate application, the application of bensulfuron-methyl to a virgin soil inhibited cellulolytic microbes, but did not do so in a soil that had received historical applications (Gigliotti et al., 1998).

Among other ALS inhibitor herbicides, similar to sulfonylureas, Imazaquin (0.14 kg/ha) also had no effect on soil microbial biomass, soil dehydrogenase, or hydrolase activity when applied to field-grown soybean (Seifert et al., 2001). In both field trials and laboratory experiments, the field rate of imazethapyr (0.05 kg/ha) had no adverse effects on the microbiological processes tested, but at 10× and 100× higher rates, the herbicide decreased dehydrogenase activity and increased hydrolyase, protease, and catalase activity (Perucci and Scarponi, 1994).

Table 5 Studies investigating the effect of sulfonylurea herbicides on soil microbial activity.

Level with respect to conventional application rate	Herbicide	Application rate (µg/kg)	Function	Effect	Duration	Soil organic carbon (%)	Soil pH	References
1×	Bensulfuron-methyl	16	Respiration	NS	—	2.3	7.7	Gigliotti et al. (1998)
1×	Bensulfuron-methyl	16	Respiration	NS	—	0.7	5.5	Gigliotti et al. (1998)
1×	Metsulfuron-methyl	10	Respiration	NS	—	1.53	6.06	Zabaloy and Gómez (2008)
1×	Metsulfuron-methyl	10	Respiration	NS	—	2.13	7.44	Zabaloy and Gómez (2008)
1×	Cinosulfuron	137	Respiration	NS	—	1.29	7.25	Sofo et al. (2012)
1×	Prosulfuron	21	Respiration	Decrease	30 d	1.29	7.25	Sofo et al. (2012)
1×	Thifensulfuron-methyl	4	Respiration	Increase	30 d	1.29	7.25	Sofo et al. (2012)
1×	Triasulfuron	14	Respiration	Decrease	30 d	1.29	7.25	Sofo et al. (2012)
1×	Nicosulfuron	300	Respiration, dehydrogenase	NS	—	1.9 ^a	7.1	Radivojević et al. (2012)

5×	Nicosulfuron	1500	Respiration, dehydrogenase	NS	—	1.9 ^a	7.1	Radivojević et al. (2012)
10×	Bensulfuron- methyl	160	Respiration	NS	—	2.3	7.7	Gigliotti et al. (1998)
10×	Bensulfuron- methyl	160	Respiration	NS	—	0.7	5.5	Gigliotti et al. (1998)
10×	Metsulfuron- methyl	100	Respiration	NS	—	1.53	6.06	Zabaloy and Gómez (2008)
10×	Metsulfuron- methyl	100	Respiration	Decrease	>40 d	2.13	7.44	Zabaloy and Gómez (2008)
10×	Cinosulfuron	1370	Respiration	Increase	30 d	1.29	7.25	Sofo et al. (2012)
10×	Prosulfuron	210	Respiration	Increase	30 d	1.29	7.25	Sofo et al. (2012)
10×	Thifensulfuron- methyl	40	Respiration	Increase	30 d	1.29	7.25	Sofo et al. (2012)
10×	Triasulfuron	140	Respiration	Increase	30 d	1.29	7.25	Sofo et al. (2012)
10×	Nicosulfuron	3000	Respiration, dehydrogenase	Increase, decrease	10 d	1.9 ^a	7.1	Radivojević et al. (2012)
10×	Triasulfuron	200	Respiration, dehydrogenase	NS	—	1.3	6.5	Dinelli et al. (1998)
10×	Primisulfuron- methyl	200	Respiration, dehydrogenase	NS	—	1.3	6.5	Dinelli et al. (1998)

(Continued)

Table 5 Studies investigating the effect of sulfonylurea herbicides on soil microbial activity.—cont'd.

Level with respect to conventional application rate	Herbicide	Application rate (µg/kg)	Function	Effect	Duration	Soil organic carbon (%)	Soil pH	References
10×	Rimsulfuron	200	Respiration, dehydrogenase	NS	—	1.3	6.5	Dinelli et al. (1998)
100×	Metsulfuron-methyl	1000	Respiration	NS	—	1.53	6.06	Zabaloy and Gómez (2008)
100×	Metsulfuron-methyl	1000	Respiration, dehydrogenase, hydrolase	NS	—	2.05	6.06	Zabaloy et al. (2008)
>100×	Bensulfuron-methyl	5000	Respiration	Decrease	1–7 d	2.49	6.64	Hou et al. (2009)
>100×	Bensulfuron-methyl	5000	Respiration	Decrease	1–7 d	2.2	4.96	Hou et al. (2009)
>100×	Triasulfuron	5000	Respiration, dehydrogenase	Increase	50 d	1.3	6.5	Dinelli et al. (1998)
>100×	Primisulfuron-methyl	5000	Respiration, dehydrogenase	Increase	40 d	1.3	6.5	Dinelli et al. (1998)
>100×	Rimsulfuron	5000	Respiration, dehydrogenase	Increase	15 d	1.3	6.5	Dinelli et al. (1998)

NS, no statistically significant effect ($P > 0.05$) relative to control (no herbicide) treatments.

^aConverted from reported organic matter %, using a factor of organic matter/organic carbon = 1.75.

5.1.4 Triazines

There is little evidence to suggest that triazine herbicides significantly inhibit microbial activity or C-cycling when applied at recommended rates. [Moreno et al. \(2007\)](#) found that atrazine only affected microbial activity (respiration, dehydrogenase activity) at levels greater than 100 mg/kg, wherein increases, rather than decreases were observed. Furthermore, atrazine applied at a conventional rate (5 mg/kg) to five different soils had no significant effect on β -glucosidase activity ([Mahía et al., 2011](#)). The related herbicide terbuthylazine (4 kg/ha) also had no effect on soil respiration on two different apple orchard soils ([Hartley et al., 1996](#)), and did not influence soil respiration or straw decomposition when applied at 10 kg/ha ([Hantschel et al., 1994](#)). In contrast, simazine (2 mg/kg) or dinoterb (1.75 mg/kg) had no short-term effects on MBC or straw turnover, but in the long term (after 33 days) both herbicides reduced degradation and respiration of added straw C ([Harden et al., 1993](#)). Conversely, atrazine (4.48 kg/ha) stimulated C and N mineralization, but it could not be determined if this was from the herbicides or the native soil organic matter ([Haney et al., 2002](#)). Similarly, [Briceño et al. \(2010\)](#) observed significantly elevated respiration from in sieved pasture soil treated with low levels of atrazine (at 1, 2, and 3 mg/kg), but only for the first 10 days after application. These examples highlight the need for temporal studies of herbicide effects on soil microbes, as well as dose responses, in order to understand potential integrated effects over longer time periods as herbicides dissipate.

5.1.5 Phenoxycarboxylic Acids

The addition of low rates of 2,4-D at 0.5 mg/kg to soil microcosms produced only minor and transitory effects on microbial respiration ([Zabaloy and Gómez, 2008](#)), and higher rates (5 mg/kg) showed transient effects on other measures of microbial activity, inhibiting hydrolase activity and stimulating dehydrogenase activity in the short-term (<24 days) ([Zabaloy et al., 2008](#)). Even higher rates of 2,4-D (50 mg/kg) were reported to reduce a larger suite of enzyme activities (acid and alkaline phosphatases, arylsulfatase, urease, protease and β -glucosidase) but all had recovered by 15 days after application, except protease ([Bécaert et al., 2006](#)). Aerobic degradation of cellulose and cellobiose were not impacted by conventional rates of bentazon or MCPA, but higher rates (100 times) reduced turnover ([Schellenberger et al., 2012](#)). Finally, triclopyr for control of woody weeds had no impact on soil enzyme activity or

substrate-induced respiration, whereas the alternative management practice of tree cutting enhanced β -glucosidase and phosphatase activity (Souza-Alonso et al., 2013).

5.1.6 Phenylureas

Niemi et al. (2009) observed negligible effects on the activity of a variety of enzymes in fallow soil treated with linuron at standard rate of 0.7 kg/ha and also at 7 kg/ha. Linuron and metoxuron applied at a range of rates (5, 50, and 500 mg/kg) only had an inhibitory effect on CO₂ evolution at 500 mg/kg, with some minor reduction also found for linuron at 50 mg/kg (Grossbard and Marsh, 1974). Linuron (4, 20, 100 mg/kg) temporarily stimulated substrate-induced respiration in dose-dependent manner (<7 days), but dehydrogenase activity was repressed at the higher rate of 100 mg/kg (Cycoń et al., 2010). Chloroxuron, diuron, fluometuron, metobromuron, and monuron added to soil at 500 mg/kg caused an initial stimulation of CO₂ production, followed by indications of inhibition (Grossbard and Marsh, 1974). Although one study found that diuron at all levels above 1.67 mg/kg reduced microbial activity (measured by microcalorimetry) in a dose-dependent manner (Prado and Airoidi, 2001), the duration of this experiment was only 2 days and it is unknown if the microbial activity rebounded after this time, making it difficult to compare to other data sets.

5.1.7 Dinitroanilines

Trifluralin reduced respiration at all doses of 16, 32, 64, and 96 mg/kg at 20°C, but no consistent effects were found at higher temperature (Aka Sağlıker, 2009). The effects of trifluralin and 12 of its soil-formed metabolites on the decomposition of radio-labeled glucose, protein, and cellulose were determined using ¹⁴CO₂ evolution from soil as a measure of decomposition. Trifluralin increased ¹⁴C-glucose mineralization rates, but these increases could be eliminated by providing additional N. Trifluralin had no inhibitory effect on the mineralization of protein or cellulose, but five of the metabolites inhibited glucose mineralization. None of the trifluralin metabolites affected protein mineralization. Seven trifluralin metabolites increased the rate of cellulose mineralization when applied at rates exceeding those that would be expected in soil. After considering the rate of metabolite application and the magnitude of the responses observed, these compounds are expected to have no major effects on the microbial population (Boyette et al., 1988)

5.1.8 Others

Most studies have found limited effects of other herbicide classes on general measures of microbial activity at conventional application rates, including pendimethalin, difenzoquat (both 0.5–5 mg/kg), or thiobencarb (2.5–25 mg/kg) (Atlas et al., 1978); mesotrione (0.45 mg/kg) (Crouzet et al., 2010); propanil (5 mg/kg) (Kyaw and Toyota, 2007); and dalapon at 2.6 or 26 mg/kg (Greaves et al., 1981). Similarly, ethofumesate did not affect dehydrogenase activity at the conventional rate of 5 mg/kg, but reduced activity at 50 mg/kg while also increasing the metabolic quotient (Muñoz-Leoz et al., 2013). Application of asulam also had no or minor effects at 16 mg/kg on C turnover, but 160 mg/kg significantly inhibited respiration of cellulose-amended soil decomposition processes in soil (Marsh, 1980).

The general trend of limited impact of herbicides at conventional rate on microbial activity is supported by a study conducted by Lewis et al. (1978). They surveyed the impact of 25 herbicides and herbicide mixtures applied at commonly used rates, finding no effects on respiration, assayed by CO₂ evolution and dehydrogenase activity, in either silty clay loam or loamy sand. Organic matter decomposition, determined by the amount of CO₂ evolved and inorganic N formed from decomposing alfalfa tissue, was also unaffected. Moreover, selected herbicides (trifluralin, linuron, dinoseb) at concentrations 100-fold higher than the recommended rates did not affect alfalfa decomposition (Lewis et al., 1978).

Nevertheless some exceptions are apparent. In one case, fluazifop-butyl application at a range of concentrations generally stimulated the decay of calico buried in herbicide-treated soil, compared to controls (Abdel-Mallek et al., 1996). Napropamide (2.25 mg/kg) reduced dehydrogenase activity when applied at conventional rates, but the duration of this effect was only 14 days (Cycoń et al., 2013b). Mesotrione application affected active chlorophyll concentrations in soil, suggesting a reduction in C-input by surface crusts (Crouzet et al., 2013), but the impact of this on the entire soil profile and plant growth is unlikely to be significant in cropping soils. More importantly, Pampulha et al. (Pampulha et al., 2007) observed that glufosinate-ammonium (1, 10, or 100 mg/kg) dramatically reduced dehydrogenase at all levels by over 50%, without recovery by the end of a 40-day soil incubation. Sessitsch et al. (2005) also found that canola receiving 3 kg/ha of glufosinate-ammonium had lower rhizosphere activities of invertase, urease and phosphatase, but only at the third sampling time, 2 months after application. A lack of studies disputing or offering explanations for the findings of Pampulha et al. (2007) and Sessitsch et al. (2005) suggests that

further work should be conducted to better understand the impacts of glufosinate on soil microbial activity.

5.1.9 Conclusions

Herbicide application can affect soil respiration, emissions of other greenhouse gases, rates of organic matter decomposition, C and N mineralization, enzyme activities, and substrate utilization patterns. What is not clear, however, is the nature of the responses of these variables to herbicide application. That is, the magnitude and direction of responses differ widely between studies. Despite this, it is clear that effects can be modulated by the identity and dose of the herbicides, the timing of application, and soil type. Standardized testing of herbicide impacts on soil biota would greatly enhance our understanding of their impacts, and may provide insights into the mechanisms that underpin these responses.

5.2 Nitrogen Cycling

5.2.1 Glycine

Data from a number of studies suggests that glyphosate applied at conventional rates has little impact on N-cycling in soil. Glyphosate had no direct effect on N-fixation when applied to different soils at rates of 2.6 kg/ha (Muller et al., 1981) or 1.25 kg/ha (Angelini et al., 2013). Furthermore, an in vitro study found no inhibition of 122 rhizobial isolates by the herbicides atrazine, glyphosate, MCPA, paraquat, imazethapyr, linuron, or metolachlor at field rates, equivalent to 3.7 kg/ha (Drouin et al., 2010). A functional dose-response study by Martensson (1993) predicted that heterotrophic or cyanobacterial N-fixation would only be inhibited at levels of 400 mg/kg or higher, representing levels of approximately 100 times the recommended dose.

Glyphosate has variable, but generally minimal effects on N-mineralization (ammonification). Stratton and Stewart (1991) found that glyphosate stimulated N-mineralization by 50% at concentrations ranging from 140 to 550 mg/kg when applied to agricultural or forestry soils, respectively. N-mineralization activity was also stimulated in forest litter that had been exposed to glyphosate during spraying. However, these rates are relatively high and may not be representative of standard practice. At more conventional levels of 10 mg/kg, Tu (1994) found that glyphosate had no impact on N-mineralization in an agricultural soil. Damin et al. (2012) found that glyphosate (1.44 kg/ha) application to a cover crop (black oat) slowed down

the mineralization of N from these plant residues, which resulted in reduced N uptake by the subsequent maize crop—but the authors suggest that this finding was related to the effect of the glyphosate on the C:N ratio of the black oat crop, rather than a direct effect on the soil microorganisms responsible for N-mineralization.

With regards to nitrification and denitrification, Muller et al. (1981) found that glyphosate application (4.2 or 18 mg/kg) to two different soils had no direct effect on either process. Stratton and Stewart (1991) also found that glyphosate applications had little effect on nitrification or denitrification, and predicted that levels of 1000–2000 mg/kg would be required to inhibit nitrification and 450 mg/kg could inhibit denitrification. These represent levels at least 100× greater than PEC after application at label rates in Australian broadacre cropping systems. Although Tu (1994) observed a slight reduction in nitrification caused by application of 10 mg/kg glyphosate, this effect was only temporary and levels of nitrate had returned to control levels 3 weeks after treatment. By contrast, Kyaw and Toyota (2007) observed that glyphosate at 2 kg/ha significantly reduced N₂O emissions by 20–90% in two different soils amended with organic matter in the form of rice straw or chitin. This result deserves further study, especially since reduced denitrification could be seen as a positive rather than negative consequence in terms of reduced greenhouse gas emissions.

5.2.2 Chloroacetanilides

The chloroacetanilide herbicides have been reported to have a mixed effect on N-cycling, depending on the specific herbicide, agricultural system, or pathway under investigation. For example, butachlor application at conventional rates of 1.5–2 kg/ha increased the number of N-fixing organisms in rice paddy soil in two different studies (Das and Debnath, 2006; Yen et al., 2013). Chen et al. (2009) used DGGE of *nifH* gene fragments to show that butachlor at 0.15 and 1.5 kg/ha caused shifts in the diversity of diazotrophs, resulting in an initial suppression and then enhancement of acetylene reduction (N-fixation). Any increase in N-fixation is unlikely to be caused by stimulation of N-fixing cyanobacteria, as Kumari et al. (2012) found that butachlor reduced N-fixation by cyanobacterial mats from multiple soils and reduced cyanobacterial diversity. Moreover, other chloroacetanilides herbicides including metolachlor and alachlor have been shown to reduce diazotroph numbers and N-fixation in aerobic soils (Angelini et al., 2013; Pozo et al., 1994). Although this discrepancy between butachlor and other

chloroacetanilides could be due to the specific differences in their chemical structure, it is equally possible that the effects of chloroacetanilides on N-fixation vary depending on the oxygen status of the soil, since butachlor is most often used for weed control in flooded rice paddies. Such a hypothesis is yet to be tested.

The effects of chloroacetanilides on other N-cycling pathways are generally quite limited. Application rates of up to 10 mg/kg of butachlor either have no effect on urease activity and N-mineralization (Wang et al., 2007, 2009; Singh et al., 2012) or temporarily stimulate urease activity (Xia et al., 2011). There is some evidence that higher concentrations (>50 mg/kg) of butachlor, alachlor, or acetochlor can inhibit urease activity (Wang et al., 2007, 2009), denitrifying bacteria (Pozo et al., 1994), or nitrifying bacteria (Li et al., 2008), respectively, but such levels are unlikely to occur in soils after single applications at recommended rates.

5.2.3 *Sulfonylureas*

Of all the herbicide classes, evidence suggests that the sulfonylureas pose the greatest risk to nitrogen cycling process (Fig. 3). Martensson (1993) reported that the lowest observable effect level (LOEC) on both heterotrophic and cyanobacterial N-fixation for a sulfonylurea herbicide, chlor-sulfuron, was 0.2 mg/kg. Repeated application of a related herbicide, chlorimuron-ethyl (0.03 kg/ha), over 5–10 years to soybean crops significantly reduced the number and diversity of N-fixing bacteria (Zhang et al., 2013). The authors demonstrated that accumulation of the herbicide had occurred as a result of its relatively long half-life, so it is possible that levels had approached or exceeded the threshold identified by Martensson (Martensson, 1993). By contrast, application of bensulfuron-methyl to two soils (one with no history of application) at rates of 0.016 or 0.16 mg/kg did not affect counts of N-fixing microbial colonies, but the rapid half-life of bensulfuron-methyl (1–3 weeks) could explain the lack of effect (Gigliotti et al., 1998).

As with N-fixation, there is evidence that sulfonylurea herbicides can impact on mineralization and nitrification at recommended or slightly higher rates. El-Ghamry et al. (2001, 2002) found that a low rate (0.01 mg/kg) of sulfonylureas metsulfuron-methyl and bensulfuron-methyl had no impact on N mineralization, but higher rates (0.1 mg/kg) transiently reduced N mineralization up to 10 days after application. Application of bensulfuron-methyl at recommended levels (0.06 kg/ha) or 10-times higher (0.5–0.6 kg/ha) significantly reduced nitrification in some soils, but usually

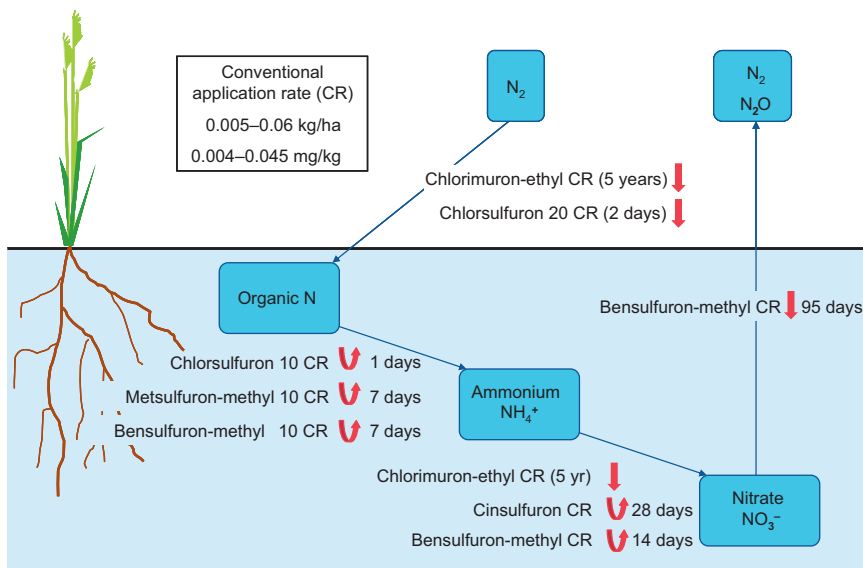


Figure 3 Documented effects of different sulfonylurea herbicides on processes involved in soil nitrogen cycling. Numbers in brackets after the herbicide name and application rate indicate repeated applications over that timeframe. Down arrows indicate decreases in function with no return to control levels within the experimental timeframe, whereas convex arrows indicate a temporary decrease in function over the time period specified after the arrow.

only temporarily (Saeki and Toyota, 2004; Gigliotti et al., 1998). A conventional rate of cinosulfuron (0.04 mg/kg) also temporarily reduced nitrification activity over a period of 1 month (Allievi and Gigliotti, 2001). Repeated application of chlorimuron-ethyl (0.03 kg/ha) over 5–10 years to soybean crops also significantly reduced the number and diversity of nitrifying bacteria, which translated to a strong reduction in nitrification potential as measured by a functional biochemical assay (Zhang et al., 2013). Finally, Das et al. (2011) found that a single dose of bensulfuron-methyl at 0.35 kg/ha also reduced N_2O emissions.

5.2.4 Triazines

The effects of triazine herbicide on N-cycling have not been studied as intensively as other high-use herbicides. Cortina et al. (2010) reported that simazine application (0.75 kg/ha) significantly reduced N fixation in biological soil crusts, but the practical impacts of this on the entire soil profile

is not clear. Simazine applied at 10 mg/kg did not affect N mineralization (Tu, 1994), but the related herbicide atrazine caused a short-term (6 weeks) inhibition of N-mineralization in five different soils when applied at 5 mg/kg (Mahía et al., 2011). A mixture of two other triazine herbicides (terbutryne 34% plus terbuthylazine 15%) at 3 kg/ha also decreased N mineralization in two soils, but increased it in one other soil (Kara et al., 2004). This herbicide mixture (Kara et al., 2004) and simazine applied at 10 mg/kg (Tu, 1994; Hernández et al., 2011) also temporarily inhibited nitrification. Higher levels of simazine (50 mg/kg) completely inhibited nitrification (in fertilized soil) by inhibiting the growth of specific ammonia-oxidising bacteria, as determined by DGGE (Hernández et al., 2011). Metribuzin also inconsistently affected nitrification in different soil types, with inhibition of nitrification in one sandy soil and one organic soil and stimulation in one clay soil; however, all effects were transient and not detected consistently over time (Junnala et al., 1993). Hexazinone at 15 mg/kg did not affect N-cycling processes but significantly affected nitrification when applied at 10 times these levels (Vienneau et al., 2004)

5.2.5 Phenoxycarboxylic Acids

Limited data are available, but 2,4-D has been shown to have some toxicity toward heterotrophic N-fixation with a lowest observable effect concentration (LOEC) at 21 mg/kg (Martensson, 1993). In contrast, cyanobacterial N-fixation was less sensitive with a LOEC of 210 mg/kg (Martensson, 1993). 2,4-D application (50 mg/kg) reduced urease and protease activity in short term but urease recovered by 15 days after application and protease was only slightly lower (Bécaert et al., 2006). 2,4-D at 2.25 kg/ha had no effect on nitrification, but 9 and 36 kg/ha showed a marked inhibition of nitrate accumulation especially during the first half of the incubation period (32 days)

5.2.6 Phenylureas, Amides

Some effects of phenylurea herbicides on N-cycling have been reported, but always at levels higher than would be expected from a recommended application rate. For example, linuron only reduced plate counts of N-fixing heterotrophs and nitrification at 400 mg/kg and had no effect at lower levels of 4, 20, or 100 mg/kg (Cycoń and Piotrowska-Seget, 2007; Cycoń et al., 2010). Other reports confirm the negligible effect of a range of phenylureas on N-cycling processes at expected environmental concentrations (<50 mg/kg), but provide additional evidence for the inhibition of

nitrification at higher levels (>50 mg/kg) (Corke and Thompson, 1970; Grossbard and Marsh, 1974; Tu, 1993).

5.2.7 Other Herbicide Classes

With regard to other herbicide classes, only a few reports have identified discernible impacts on N-cycling at conventional rates. Napropamide significantly reduced plate counts of nitrifying and N-fixing bacteria, as well as net nitrification, at conventional (2.25 mg/kg) and higher (22.5 mg/kg) rates, and none of these measures had rebounded to control levels after 28 days (Cycoń et al., 2013b). Other herbicides, including bentazon applied at 10 mg/kg (Allievi et al., 1996), and ethofumesate at 5 mg/kg (Muñoz-Leoz et al., 2013), did not affect N-cycling processes but significantly affected nitrification when applied at 10 times these levels.

5.3 Cycling of P and Other Elements

Herbicide application to plants or soils may impact on P acquisition by plants by directly influencing plant metabolism or via alterations to the soil microbial and/or fungal communities that have been implicated in either P-cycling or P uptake by plant roots. Herbicide impacts on plant vigor and root growth due to general toxicity of the herbicide on plants, or specific root pruning effects such as those observed following sulfonylurea herbicide application (Rengel and Wheal, 1997; Robson and Snowball, 1990), can reduce the uptake of a range of soil immobile nutrients due to reduced root exploration of the soil volume. While these impacts are important considerations in managing plant nutrition, they are not discussed in detail here as the focus of this review is the impact of herbicides on soil biology and function.

The two major mechanisms by which herbicides can influence soil biological processes involved in P uptake by plants are: (1) perturbation of microbial/fungal communities involved in organic P turnover in soils and (2) potential reduction of root colonization, or performance, of mycorrhizal fungi that are involved in P uptake in many crop species.

5.3.1 Organic P Turnover

Most studies investigating the effects of herbicides on P turnover in soils have quantified soil acid and/or alkaline phosphatase enzyme activities to assess the capability of a soil to maintain its capacity to mineralize organic P forms into the inorganic P species that are absorbed by plant roots. Studies that provided adequate information are summarized in Table 4, but differences in herbicides, application rates, soil type, and experimental conditions make it

difficult to draw firm conclusions about the results. Moreover, studies with the same herbicide (eg, butachlor) often had contrasting results (Table 6). Even within any given study it is difficult to draw logical conclusions: for example, Perucci et al. (2000) found a significant decline in acid and alkaline phosphatase activity at 7 days (10–15%) at 5 mg/kg soil yet at 50 mg/kg soil there was a significant increase in alkaline phosphatase activity at 7 days (5–10%) but no change in acid phosphatase activity.

Das and Debnath (2006) examined the impact of four herbicides on populations of phosphate-solubilizing organisms in the rhizosphere of rice plants under field conditions. Significant increases in the populations of phosphate-solubilizing organisms in the rhizosphere of rice plants following applications of butachlor, oxadiazon, and oxyfluorfen at their recommended field rates (2.0, 1.5, 0.4, and 0.12 kg/ha, respectively) were observed. However, because weeds were not controlled by hand, the reduction in weed competition in plots receiving herbicide led to larger plants and increased yields. Despite the conclusion of the authors, we contend that it is not possible to determine whether the increase in P-solubilizing organisms in the rhizosphere was specifically due to the herbicides or due to greater availability of carbon in the rhizosphere in the larger plants.

5.3.2 *Mycorrhizas*

Fungicides are known to inhibit mycorrhizas but the impact of herbicides on mycorrhizal colonization and survival is not well understood. Most studies have focused on ectomycorrhizae because of their importance in silviculture or arbuscular mycorrhizas (AM) because they colonize a wide range of agriculturally important plants (Cavagnaro, 2008). Many studies on herbicide-mycorrhiza interactions have been conducted in laboratory conditions and the results have varied from stimulated growth to suppression of growth depending on the rate of active ingredients used and specific fungal species investigated (reviewed by Trappe et al., 1984).

The results of field and greenhouse studies are also variable: for example, studies investigating a range of herbicides including trifluralin, alachlor, diazinon, triclopyr, imazapyr, chlorsulfuron, sulfometuron, and glyphosate at a range of rates up to 2× field rates found no effect on colonization of roots by arbuscular mycorrhizal fungi (AMF) or ectomycorrhizal fungi of crop plants or pine trees, respectively, even when plant growth was retarded (Burpee and Cole, 1978; Busse et al., 2004; Chakravarty and Chatarpaul, 1990; Mujica et al., 1999; Pasaribu et al.,

Table 6 Impact of herbicides on phosphatase activity in soils from published laboratory incubation studies.

References	Soil type	Herbicide	Application rate (mg/kg)	Timeframe	Impact on phosphatase activity	Comments
Perucci et al. (2000)	Vertic Aquic Ustorthent	Rimsulfuron	5	30 d	Significant decline in acid and alkaline phosphatase activity at 7 d (10–15%) but no differences at 14 and 30 d	
			50	30 d	No effect on acid phosphatase activity but caused a significant increase in alkaline phosphatase activity, but only at 7 d (5–10%)	
		Imazethapyr	16.7	30 d	Acid phosphatase activity declined significantly at 7 and 30 d (around 30%) but not at 14 d, while alkaline phosphatase activity declined significantly at 14 and 30 d (5–10%)	
			167	30 d	Significant increase in acid and alkaline phosphatase activity at 7, 14, and 30 d (20–60%)	
Cycoń et al. (2013b)	Orthic Luvisol	Napropamide	2.25	28 d	Significantly reduced acid and alkaline phosphatase activity by around 5 and 10%, respectively, at 1 d after application but no difference by 14 d after application	
			22.5	28 d	Significantly reduced acid and alkaline phosphatase activity by around 20 and 40%, respectively, at 1 d after application, and these differences were sustained at 14 and 28 d after application	

(Continued)

Table 6 Impact of herbicides on phosphatase activity in soils from published laboratory incubation studies.—cont'd.

References	Soil type	Herbicide	Application rate (mg/kg)	Timeframe	Impact on phosphatase activity	Comments
Sofo et al. (2012)	Vertic Ustorthens	Cinosulfuron	137	30 d	No reduction in alkaline or acid phosphatase activity at 30 d	Rates in paper were given as 350, 55, 10, and 37 g/ha for cinosulfuron, prosulfuron, thifensulfuron methyl, and triasulfuron, respectively. The authors did not state the actual amounts applied to the incubated soils but stated that the rates were calculated assuming an even distribution of the herbicides in the 0–20 cm layer (bulk density of 1.28 g/cm ³). We have calculated rates in µg/g soil based on these numbers and have assumed that the rates referred to g of active ingredient.
			1370	30 d	Significant (around 20%) reduction in both alkaline and acid phosphatase activity at 30 d	
		Prosulfuron	55	30 d	No reduction in alkaline or acid phosphatase activity at 30 d	
			550	30 d	No reduction in acid phosphatase activity, but significant increase in alkaline phosphatase activity (5%) at 30 d	
		Thifensulfuron methyl	10	30 d	Significant increase in alkaline (around 7%) and acid (around 12%) phosphatase activity at 30 d	
			100	30 d	Significant increase in alkaline (around 7%) and acid (around 5%) phosphatase activity at 30 d	

Wang et al. (2007)	Phaeozem	Triasulfuron	37	30 d	Significant increase in alkaline phosphatase activity (around 2%) but no effect on acid phosphatase activity at 30 d	We have assumed concentrations given referred to active ingredient.
			370	30 d	Significant increase in alkaline (around 5%) and acid (around 10%) phosphatase activity at 30 d	
	Phaeozem	Butachlor	5	28 d	No significant effect on acid phosphatase activity	
			10	28 d	Significant reduction (around 20%) in acid phosphatase activity	
			50	28 d	Significant reduction (around 60%) in acid phosphatase activity	
	Not stated	Butachlor	50	28 d	No significant effect on acid phosphatase activity	We have assumed concentrations given referred to active ingredient.
Wang et al. (2009)			100	28 d	Significant reduction (around 30%) in acid phosphatase activity	

(Continued)

Table 6 Impact of herbicides on phosphatase activity in soils from published laboratory incubation studies.—cont'd.

References	Soil type	Herbicide	Application rate (mg/kg)	Timeframe	Impact on phosphatase activity	Comments
Xia et al. (2011)	Not stated	Butachlor	5	21 d	Reduction in acid phosphatase activity of about 5% after 1 d to about 8% after 21 d, but no significance was tested	We have assumed concentrations given referred to active ingredient.
			10	21 d	Reduction in acid phosphatase activity of about 7% after 1 d to about 20% after 21 d, but no significance was tested	
			50	21 d	Reduction in acid phosphatase activity of about 10% after 1 d to about 30% after 21 d, but no significance was tested	
			100	21 d	Reduction in acid phosphatase activity of about 25% after 1 d to about 35% after 21 d, but no significance was tested	
Rasool et al. (2014)	Not stated	Butachlor	23	35 d	Under flooded conditions alkaline phosphatase activity was significantly reduced at 28 d but was not different to the control at 14, 21, and 35 d. Under aerobic conditions alkaline phosphatase activity was significantly increased at 14, 21, and 28 d, but no effect by 35 d	Herbicide rates were applied at 23 µg AI/g soil, 230 µg AI/g soil, and 23 mg AI/g soil representing 1×, 10× and 100× field rates, respectively where 1x is the equivalent 1.5 kg active ingredient/ha

230	35 d	Under flooded conditions alkaline phosphatase activity was significantly higher at 14 d, significantly lower at 21 and 28 d, and not different to the control at 35 d. Under aerobic conditions alkaline phosphatase activity was significantly higher than control at 14 and 28 d and not significantly different from control soil at 21 and 35 d	assuming a uniform distribution of the chemical in the top 0–10 cm of soil (bulk density of 1.3 g/cm ³). However, the rate of 25 mg AI/g soil is actually 1000× field rate and we presume that 25 mg AI/g soil was actually added rather than the true 100× rate.
2300	35 d	Under flooded conditions alkaline phosphatase activity was significantly higher at 14 and 21d, significantly lower at 28 and not different to control soil at 35 d. Under aerobic conditions alkaline phosphatase activity was not significantly higher than control at 14 and 35 d and but was significantly higher than control soil at 21 and 28 d	

2013). In contrast, glyphosate at 0.8 and 3 mg/kg reduced mycorrhizal spore counts when applied directly to the soil (Druille et al., 2013a,b) but had no effect when applied to plant foliage (Druille et al., 2013b). The same authors found reduced root colonization by AMF when the plants were treated with glyphosate, which coincided with a reduction in photosynthetic capacity of the plants. Given that no reduction in AM colonization was found in glyphosate-resistant soybean roots when the plants were treated with glyphosate (Mujica et al., 1999; Powell et al., 2009), this may suggest that the reduced colonization was due to a reduction in photosynthate supply to roots rather than a direct inhibitory effect of glyphosate on the AMF. Similar questions surround the results of Ramos-Zapata et al. (2012): paraquat reduced AM colonization in maize roots when used over a 13-year period in comparison to other cover crops and mulch treatments, but there is no indication of whether this was a direct effect of paraquat or due to reduced carbon inputs to soil for 13 years in the interrow where paraquat was sprayed.

5.4 Pathogens and Disease Incidence

The interaction of herbicides, pathogens, and crop plants has the potential to either increase or decrease the incidence of disease and subsequent yield decline through a number of mechanisms (Fig. 4). Kortekamp (2011) recently reviewed these interactions and the reader is directed to this review for a more in-depth discussion, particularly with respect to the herbicides glyphosate and glufosinate. We briefly summarize the pertinent findings of Kortekamp (2011) later in the chapter and provide an update in terms of more recent literature and additional detail on some other herbicide classes.

5.4.1 Glycine

As highlighted by Kortekamp (2011), the application of glyphosate in vitro can inhibit a variety of soil-borne pathogens, including *Sclerotium rolfsii* (Westerhuis et al., 2007), *Pythium ultimum*, and *Fusarium solani* f. sp. *pisi* (Kawate et al., 1992), *F. solani* f. sp. *glycines* (Sanogo et al., 2000), *Nectria galligena* (Burgiel and Grabowski, 1996) and *Rhizoctonia solani* (Lancaster et al., 2008). However, the effect of glyphosate in vitro appears to have little relevance to the severity of disease in whole-plant bioassays or in the field. In fact, increased disease severity after glyphosate application has been observed in sugarbeet inoculated with *R. solani* and *Fusarium oxysporum* (Larson et al., 2006); sugarcane infected with *Pythium arrhenomanes*

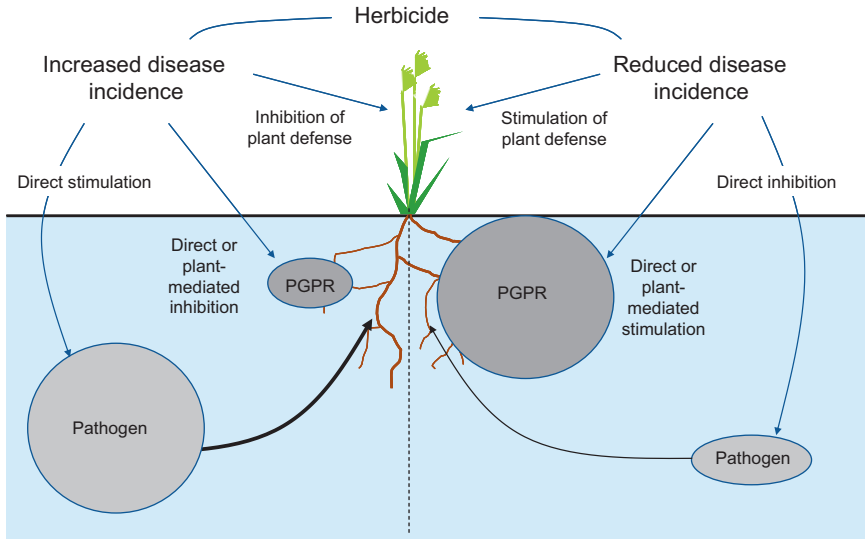


Figure 4 Potential mechanisms by which herbicides can impact the incidence of disease in cropping systems. PGPR, plant growth promoting rhizobacteria.

(Dissanayake et al., 1998); soybean infected with *Phytophthora megasperma* f. sp. *glycinea* (Keen et al., 1982), bean with *Colletotrichum lindemuthianum* (Johal and Rahe, 1990), tomato with *Fusarium* spp. (Brammall and Higgins, 1988), and grapevine with *Cylindrocarpum* sp. (Whitelaw-Weckert, 2010).

Since glyphosate inhibits the synthesis of aromatic amino acids that are a key component of many plant defense compounds, increased disease could occur via indirect effects of the herbicide on plant health or pathogen resistance, which subsequently allows for greater colonization and/or pathogenesis by the disease-causing organism (Kortekamp, 2011). This is supported by the observation that glyphosate application increased tissue levels of shikimate (the precursor to aromatic amino acids) even in glyphosate-resistant wheat varieties (Larson et al., 2006). It should be borne in mind that this effect can also occur in weed species, leading to additional agronomic considerations. For example, glyphosate increased *Fusarium* density in both glyphosate-sensitive and resistant types of the weed species *Amaranthus rudis* (Rosenbaum et al., 2014), and in this sense could assist in speeding-up weed control (Baley et al., 2009). However, increasing the susceptibility of either crop or weed species may also accelerate the buildup of pathogen density over time, leading to a higher risk of pathogen outbreak in nonresistant crops.

In other cases, as [Kortekamp \(2011\)](#) points out, there also is evidence that glyphosate can upset the balance of the soil microbial community and reduce the innate suppressiveness of the soil to pathogen dominance. For example, glyphosate (at 0.8, 1.2, and 2.4 kg/ha) inhibited the growth of *Pseudomonads* and indole-acetic acid producing microorganisms, concomitant with increasing *Fusarium* infection in soybean roots in a dose-dependent manner ([Zobiolo et al., 2011](#)).

5.4.2 Chloroacetanilides

Acetochlor application at rates of 50–250 mg/kg significantly altered the structure of soil fungal communities, with a temporary increase in pathogens and reductions in common nonpathogens ([Xin-Yu et al., 2010](#)). Increasing acetochlor concentrations (50, 150, 250 mg/kg) also reduced the number and diversity of culturable *Pseudomonads* showing antagonism toward *Rhizoctonia* ([Wu et al., 2009](#)). Since whole-plant assays were not conducted, it is difficult to speculate on whether these effects would translate to increased plant disease, but these results certainly suggest an increase in the risk of pathogen infection.

5.4.3 Sulfonylureas and Other ALS Inhibitors

In one of the earliest studies on the interaction of herbicides with disease in cereal crops, [Rovira and McDonald \(1986\)](#) followed up on field observations of farmers and agronomists who observed high incidences of poor barley growth—with symptoms typical of root rot—in alkaline soils treated with chlorsulfuron in the previous season. Through controlled-environment experiments with *R. solani* they found that chlorsulfuron at the equivalent of 2.5 g/ha (0.004 mg/kg) significantly increased root disease caused by *R. solani* in wheat and barley, but chlorsulfuron did not increase the incidence of the disease take-all, caused by *Gaeumannomyces graminis* var. *tritici*, in wheat ([Rovira and McDonald, 1986](#)). [Lee et al. \(2012\)](#) also found that sublethal doses (<20% recommended rate) of two other ALS-inhibitor herbicides, imazamox, and propoxycarbazone-Na, reduced barley growth and increased *R. solani* disease symptoms. The authors suggested that sublethal rates of herbicides and *R. solani* could alter the severity of injury symptoms, possibly owing to the herbicide predisposing the plant to the pathogen ([Lee et al., 2012](#)). Similar results have also been observed for soybeans. [Bradley et al. \(2002\)](#) found that the ALS-inhibitor imazethapyr increased the severity *Rhizoctonia* root and hypocotyl rot of soybeans compared to the no-herbicide control in a number of different environments.

Some cultivars were clearly more susceptible than others to this interaction. Long-term (5 or 10 year) application of chlorimuron-ethyl to soybean fields of near-neutral pH have also been observed to increase the prevalence of *F. oxysporum*, *R. solani*, and *Phytophthora sojae* (Zhang et al., 2011). In contrast, field application of chlorsulfuron to wheat growing in acidic soils reportedly has little impact on the severity of *Rhizoctonia* root rot (Wong et al., 1993). These results strongly suggest that farmers using sulfonylurea or other ALS-inhibiting herbicides should monitor crops growing in fields previously treated with ALS-inhibitors for disease symptoms, particularly in alkaline soils where herbicide residues are more likely to persist.

5.4.4 Triazine and Other PSII Inhibitors

When propazine was used continuously for 5 years as a preemergent herbicide in a large *Pinus radiata* nursery where the soils were not believed to be conducive to *Phytophthora cinnamomi* root rot, disease appeared after 2 years and rapidly increased in intensity despite attempts to control it with fungicides. The disease and the fungus virtually disappeared within one season when chlorthal dimethyl replaced propazine (Marks and Cerra, 1991). Follow-up experiments found that propazine increased the number of spore-forming bacteria, which appeared to stimulate sporangia formation by *P. cinnamomi*, and that the chemical may also have damaged root tissue and increased root susceptibility to infection. By contrast, chlorthal dimethyl had a negative effect on all microbiota and helped suppress *P. cinnamomi* (Marks and Cerra, 1991). It would be of interest to see if other triazine herbicides have similar effects, and to examine these potential mechanisms in other plant-pathogen systems.

In contrast, research into another PSII inhibitor, metribuzin, found that it had no effect on the five selected plant-growth promoting organisms at conventional and higher rates (Myresiotis et al., 2012), and even stimulated the growth of the pathogen-inhibitory strain *Streptomyces corchorusii* (El-Shanshoury et al., 1996). The combination of metribuzin with the *Streptomyces* strain inhibited *Fusarium* and decreased disease incidence in tomatoes (El-Shanshoury et al., 1996).

5.4.5 Other Herbicides

Three protoporphyrinogen oxidase inhibitor herbicides—azafenidin, sulfentrazone, and flumioxazin—were investigated for their effects on *Pythium* root rot in sugarcane. All three herbicides inhibited the in vitro

mycelial growth of *P. arrhenomanes*, *Pythium aphanidermatum*, and *P. ultimum*, but the effects in soil were inconsistent (Daugrois et al., 2005).

The application of diquat + paraquat, glyphosate, and trifluralin (all at 1 mg/kg) to unsterilized field soil increased take-all caused by the fungus, *G. graminis* var. *tritici* Walker by 13.0, 16.6, and 10.8% respectively, while no effect on disease was recorded in sterilized soil treated with the same herbicides. The herbicides tested had no effect on the saprophytic growth of the pathogen with the exception of glyphosate, which increased pathogen growth in unsterilized soil. The application of diquat + paraquat and glyphosate to unsterile soil had no effect on the number of actinomycetes. The diquat + paraquat treatment, however, increased the population of fungi while the glyphosate decreased the number of bacteria. The proportion of soil fungi antagonistic to the pathogen was reduced in glyphosate-treated soil. Dicamba, chlorsulfuron, and chlorthal dimethyl did not influence pathogenicity (Mekwatanakarn and Sivasithamparam, 1987)



6. ADDITIONAL CONSIDERATIONS FOR IMPACTS WITHIN AGRICULTURAL SYSTEMS

6.1 Active Ingredient Versus Formulation

Commercial products may contain a wide variety of substances in addition to the active ingredient in order to improve stability, mixing, dilution, and application (Tominack, 2000). Some examples include solvents, surfactants, emulsifiers, dispersants, binders, wetting agents, fillers, preservatives, or other compounds with specific functions (Tominack, 2000). One of the more common hurdles in ecotoxicology is extrapolating the effects of an active ingredient (eg, glyphosate) to the effects of a formulated product (eg, Round Up). Systematic toxicological assessment of every component found within the range of herbicide products is too costly and time consuming; thus, researchers are usually restricted to comparing the active ingredient against one or more commercial formulations. Studies of this nature are reasonably common in human and aquatic toxicology, but those focused on risks of herbicides to soil biota are relatively rare.

In one study, the herbicide formulation Callisto had about a 30% greater effect than its active ingredient mesotrione in reducing chlorophyll concentrations and decreasing the diversity of cyanobacterial populations in soil (Crouzet et al., 2013). In another study, soil treated with the formulated

herbicides penoxsulam or sulcotrione were more frequently avoided by earthworms than the corresponding rates of the active ingredients (Marques et al., 2009). Salminen et al. (1996) conducted dose–response experiments for terbuthylazine and its formulation Gardoprim. Terbuthylazine had no toxic effects on the soil animals tested (microbes, oppioid mites, two gamasid mite species, enchytraeids, and nematodes), whereas Gardoprim had acute toxic effects on enchytraeids, with a no-observed effect level (NOEL) of 10 kg/ha, and both gamasid mites (NOEL 24 and 50 kg/ha). These three studies each suggest that formulated herbicide products are generally more toxic than their corresponding active ingredient. Although this concurs with the general findings from other ecotoxicological studies, there are always exceptions to this rule. For example, the formulation of glyphosate had marginal and inconsistent effects on the growth of five bacterial species (some isolated from soil) in vitro relative to the active ingredient (Sihtmäe et al., 2013).

6.2 Herbicide Mixtures

Herbicide mixtures are often used for weed control in order to target multiple weed species with a single application. The sheer number of possible combinations of different herbicides makes an assessment of the toxicity of specific mixtures extremely difficult. Only very few studies have attempted to assess the toxicity of herbicide mixtures as compared with individual active ingredients. Das et al. (2011) found that single doses of bensulfuron-methyl or pretilachlor alone at conventional rates reduced both N_2O and CH_4 emissions; but when applied together, this effect was absent or was reversed. Lupwayi et al. (2009) also examined the effect of numerous herbicide combinations on bacterial diversity and substrate utilization profiles in canola cropping systems. They found a number of significant differences, but could not make any general conclusions about why particular herbicide combinations caused greater disturbances.

6.3 Chronic Effects of Repeated Applications

One of the biggest concerns for landholders and scientists is the possibility of a gradual decline in soil quality caused by long-term, repeated herbicide applications (Barman et al., 2014; Strom, 2013). Biological shifts may not be detectable in short-term laboratory or glasshouse experiments and a lack of control and investment in long-term field studies means potential effects may be overlooked or misattributed to other factors. Despite these difficulties,

some data documenting the chronic effects of repeat applications are available.

To date, there is little evidence to suggest that long-term, repeat applications of glyphosate to soil causes negative shifts in soil microbial communities or functions. Biederbeck et al. (1997) found that long term (21 years) of glyphosate or paraquat application in a wheat-fallow rotation had no deleterious effects on soil microbial populations (bacteria, actinomycetes, fungi, nitrifiers, denitrifiers), nor on microbial biomass or potential C or N mineralization. Long-term field monitoring of repeated glyphosate applications (9–13 years) at 3 kg/ha to pine plantations on three different soil types also revealed no detectable effects on basal respiration, metabolic quotient, total bacteria, metabolic diversity, or mineralizable N (Busse et al., 2001). More recent investigations did not detect significant changes to microbial community structures in unplanted microcosms receiving six applications of glyphosate over 6 months (Lane et al., 2012) or in maize fields receiving annual glyphosate applications of 0.72 kg/ha over 3 years (Barriuso et al., 2011a). Perie and Munson (2000) observed that annual glyphosate (2 kg/ha) applications for 4 years reduced soil organic C by 46%, total N by 15%, and acid phosphatase activity by 64% in a forestry soil; but concluded that these impacts likely represented an indirect effect of reduced weed growth in topsoil.

There is some evidence to suggest that long-term applications of atrazine can induce significant changes in the microbial population. Atrazine applied at 4 kg/ha annually for 24 years permanently reduced the number of anaerobic bacteria, spore-formers, and cellulolytic microorganisms, and temporarily reduced the nitrifying, amylolytic and denitrifying microbial groups. Atrazine also temporarily enhanced a number of ammonifying and proteolytic organisms and permanently increased the number of *Azotobacter*. As a result of the long-term elimination of the direct vegetative cover and the concomitant loss of organic matter in the atrazine-treated soil, the phosphatase, saccharase, β -glucosidase, and urease activities of this soil were reduced by 50% or more (Voets et al., 1974). Seghers et al. (2003) also found shifts in the bacterial community structure in soil under a maize monoculture after 18 years of annual application of atrazine (0.75 kg/ha) and metolachlor (2 kg/ha). Targeted 16SrDNA PCR-DGGE showed that herbicide-treated soil had a similar structure of *Acidobacteria*, ammonia oxidizers, and actinomycetes, but three methanotrophic phylotypes were absent in chronically exposed soils. Interestingly, q-PCR and functional assays showed that the abundance and

activity of methanotrophs was not affected, suggesting a functional resilience even though community structural changes occurred. An additional study from the same plots showed that long-term atrazine application had not affected the endophytic bacterial community within the maize roots (Seghers et al., 2004). Long-term (7 years) field trials showed that soil from atrazine-treated maize plots had significantly higher microbial biomass carbon than most other plots in the final year of treatment, apparently due to increased levels of atrazine-tolerant weed spp. dominating the plots (Wardle et al., 1999). In the same trial mentioned earlier, herbicides (atrazine or sulfonylureas) did not exert any consistent detrimental effects on nematode communities and the nematode fauna in the herbicide-treated plots tended to have greater diversity (as indicated by the Shannon–Weiner index) than that in many of the other plots. Since effects were only apparent after at least 3 years, the authors recommended that to evaluate the relative effects of different agricultural practices in the long term it is necessary to sample until the ecosystem has achieved some degree of equilibrium rather than monitoring only initial cropping cycles (Yeates et al., 1999). In a similar study on the impacts of ground vegetation management strategies in a kiwifruit orchard on the composition and functioning of the soil biota, most of the results could be explained by the fact that differences in the amount of basal resources were likely to be present, rather than other components of intensification such as cultivation or herbicide (simazine plus glyphosate) application (Wardle et al., 2001).

There is also evidence that repeat applications of sulfonylurea herbicide may impact on soil microbial communities, particularly those involved in N-cycling. Long-term (5 or 10 years) repeated application of chlorimuron-ethyl significantly reduced the number and diversity of N fixing and nitrifying bacteria. Denitrifiers were also reduced in number but increased in diversity. Higher herbicide residues were also detected in plots of repeat application, suggesting incomplete dissipation over a cropping season could be an indicator of chronic risks. Long-term (5 or 10 years) application of chlorimuron-ethyl to soybean fields also significantly reduced culturable bacteria and actinomycetes, but increased fungal counts. Application of chlorimuron-ethyl increased the prevalence of *F. oxysporum*, *R. solani*, and *P. sojae* (Zhang et al., 2011). Forestry plots receiving sulfometuron-methyl (0.057–0.113 kg/ha) treatments annually for 4 years had higher inorganic N, and extractable P, than the other treatments, suggesting higher mineralization rates without consequent immobilization, for example, by weeds in control plots (Arthur and Wang, 1999)

Only a single study reporting the effects of repeat applications of phenylurea herbicides could be found. In that study, annual application of diuron (2 kg/ha), diuron plus linuron (2 + 3 kg/ha), and chlorotoluron (5 kg/ha) for 10 years to an orchard significantly reduced culturable heterotrophs and altered the soil bacterial community structure as measured by 16s rDNA DGGE and CLPP (El Fantroussi et al., 1999). These shifts in community structure equated to a loss, rather than a gain, in species diversity in all herbicide-treated plots.

Data on the effects of repeated applications for other herbicide classes are also scarce. With respect to phenoxy-acid herbicides, five applications per year of 2,4-D (4.5 kg/ha) over a 4-year period decreased culturable bacteria in soils, but had no significant effect on culturable fungi or actinomycetes (Breazeale and Camper, 1970). However, Duah-Yentumi and Johnson (1986) found that repeat application of another phenoxy herbicide, MCPA, at a rate of 1.68 kg/ha for 22 years, reduced the number of culturable actinomycetes but had no effect on other microbial groups, including aerobic and anaerobic bacteria, yeasts or fungi.

Similar inconsistencies have been found for repeat applications of dinitroaniline herbicides. Trifluralin applied annually for 4 years at 1.12 kg/ha decreased bacterial and fungal colonies but increased actinomycete counts (Breazeale and Camper, 1970). Conversely, Moorman and Dowler (1991) observed that repeat applications over 7 years of trifluralin (0.56 kg/ha) or alachlor (2.24 kg/ha) to soybean or maize monocultures, or both sequentially in crop rotation, did not have any consistent or lasting effects on culturable microorganisms. Moreover, the herbicide-treated crops did not suffer from yield decline.

Repeated applications of herbicide mixtures to a canola (Glufosinate plus Clethodim)-barley (Tralkoxydim, Bromoxynil plus MCPA) rotation in Canada showed no significant effects in the first 2 years on MBC, CLPP diversity, or β -glucosidase activity. A positive effect on diversity was observed in the third year in canola, but minor negative effects on MBC, CLPP diversity, or β -glucosidase were observed in some soils in fourth year (Lupwayi et al., 2010). The authors suggest cumulative effects may be more important, but complex experiment design makes it difficult to determine.

Overall, it is difficult to make general conclusions about the long-term effect of repeated herbicide applications. As Zhang et al. (2011) proposed, the evolution of chronic effects after repeat herbicide applications could result from a buildup of herbicide residues. This hypothesis is supported by data from the study of Baxter and Cummings (2008). They found that

repeated applications of bromoxynil (three applications each month) at 10 mg/kg fostered bromoxynil breakdown and did not significantly affect diversity, but repeat applications of 50 mg/kg inhibited breakdown and dramatically altered diversity as measured by DGGE. This suggests a threshold beyond which toxicity buildup may occur.

6.4 Comparison Against Other Weed Control Systems

Since some form of weed control is commonplace to prevent yield loss in cropping systems, a fair assessment of the effect of herbicides on soil biota should also account for the potential impacts of other weed management strategies. The primary alternative to herbicides for weed control is the use of tillage.

Herbicide-treated plots growing maize or asparagus were not significantly different to hand-hoed or cultivated plots in terms of respiration or turnover of organic amendment (ryegrass litterbags) (Yeates et al., 1999; Wardle et al., 1993). In a 21-year field experiment, Biederbeck et al. (1997) compared the impacts of a zero-tillage system using the herbicides glyphosate or paraquat against a conventional tillage system. They found that the zero-till/herbicide system had no long- or short-term effects on soil microbial populations or C or N-mineralization, whereas conventional tillage had a negative impact on most soil characteristics. Similarly, Carter et al. (2007) found that glyphosate effects on soil biological properties in a 3-year potato rotation were periodic, inconsistent, and considered to be ecologically negligible compared to greater effects of tillage on soil structure. These authors further speculated that the periodic reductions in microbial activity were related to reduced plant biomass rather than direct effects of the herbicide on soil biota.

Simpfendorfer et al. (2002) also analyzed yield declines in wheat under direct drilling with herbicides as compared to conventional tillage, and found that reduced yields were not related to herbicides but to root-inhibitory pseudomonads prevalent in undisturbed soil as compared with cultivated soil. In a broad assessment of the effect of herbicides relative to other management practices, Steenwerth et al. (2002) used multivariate analysis to analyze the microbial community profiles associated with different agricultural soils. They found that herbicide use was correlated with particular microbial PLFAs, but that these PLFA signatures were also strongly associated with fertilizer use and cultivation, suggesting an overall system effect rather than a specific herbicide-induced effect.

Overall, these results highlight the importance of considering the effects of herbicide use within a systems context. Although a particular herbicide may

have not have a direct effect, per se, on soil biology and function, a no-till system within which it is used may result in large shifts in microbial communities compared to a soil that was previously cultivated on a routine basis.

6.5 Toxicity of Herbicides Versus Other Agricultural Inputs

Although their use is increasing, herbicides represent only a part of the overall chemical inputs into most agricultural systems. It is therefore also of interest to compare the impacts of herbicides with those of other crop protection chemicals and fertilizers.

A number of studies directly comparing the nontarget effects of herbicide with insecticides and fungicides have found that fungicides generally have more adverse impacts on soil microbiota. For example, [Kumar et al. \(2012\)](#) showed that while the herbicide pretilachlor increased MBC and respiration, the insecticides chlorpyrifos and cartap and the fungicide carbendazim inhibited respiration. An in vitro study found that fungicides (especially captan and mancozeb) were generally more toxic to rhizobial isolates than the herbicides atrazine, glyphosate, MCPA, paraquat, imazethapyr, linuron, or metolachlor, as well as a number of insecticides ([Drouin et al., 2010](#)). Compared with the herbicide linuron, a fungicide mixture of mancozeb plus dimethomorph dramatically reduced counts of fungi and N-cycling bacteria ([Cycoń and Piotrowska-Seget, 2007](#)). In another toxicity study, increasing concentrations up to 500 mg/kg showed no effect of chlorsulfuron or MCPA on respiration, while the fungicide propiconazole reduced respiration by 50% when applied at 500 mg/kg ([Ahtaiainen et al., 2003](#)). [Itoh et al. \(2003\)](#) also found that a herbicide mixture (daimuron plus bensulfuron methyl) did not affect community-level physiological profiles (Biolog) at 1, 10, and 50 times the conventional application rate, but a fungicide mixture of isoprothiolane plus flutolanil at 50 times the conventional application rate altered the microbial community for up to 1 month before recovery. Fungicides have also exhibited higher toxicity to soil mesofauna than herbicides. In one study, the fungicides benomyl and carbendazim were shown to induce avoidance behavior in *E. albidus* at lower concentrations than observed for the herbicide phenmedipham ([Amorim et al., 2005](#)).

Few studies have directly attempted to assess the effects of biocides, including herbicides, against the potential impacts of fertilizer inputs. [Seghers et al. \(2005\)](#) observed that fertilizer (organic vs. mineral) had a much greater impact than herbicides (mixture of nicosulfuron, atrazine, and dimethenamide) on the abundance of methanotrophs and methane

oxidation rates. However, although larger changes were apparent, it is difficult to assess these changes as being positive or negative with respect to agricultural productivity or environmental sustainability. Muñoz-Leoz et al. (2012) found that the herbicide ethofumesate had a lower effect compared to an insecticide and fungicide at the same rate, and also compared with fertilizers (NPK and compost). According to their overall soil quality measure, these authors concluded that NPK fertilizer caused the biggest decline in soil quality.



7. CONCLUSION AND FUTURE RESEARCH NEEDS

It is clear that the impact of herbicides on soil biology and functionality is a complex issue. Although herbicides can be grouped according to chemical structure and mode of action, this does not guarantee that they will have similar impacts on soil organisms. Overall, the majority of papers reported negligible impacts of herbicides on soil microbial communities and beneficial soil functions when applied at recommended field-application rates. This is in contrast to more frequent reports of altered population dynamics and microbial activities in soils receiving herbicide inputs of 5–100× recommended rates. Even still, in the majority of cases where negative effects were observed such effects usually only lasted for periods of less than 1–2 months, demonstrating structural and functional resilience to herbicide-induced disturbance. Furthermore, we found a large amount of variability and inconsistency between different studies on the same herbicide, implying that analytical methodologies and site-specific variables, such as soil type, climate, and soil biology, strongly influence the findings of each study.

Nevertheless, some exceptions to these general trends were apparent and these require further attention from scientists and farmers alike:

- There is some evidence that glyphosate and atrazine may disrupt the feeding behavior and ecology of certain groups of earthworms, but it is unclear as to the relevance of these laboratory-based findings to field situations where their mobility would allow them to avoid undesirable herbicide concentrations;
- There is also evidence that some sulfonylurea herbicides can inhibit processes involved in N-cycling, and may thereby reduce plant-available N. This appears more likely to occur in alkaline soils where sulfonylurea degradation is slower or in instances where sulfonylurea herbicides are repeatedly applied, resulting in a buildup of residues. Considering that the imidazolinone herbicides act in the same manner, by inhibiting ALS

synthase, attention should also be given to monitoring the potential impacts of this herbicide class;

- A number of reports show that certain herbicides (eg, glyphosate, propazine, sulfonylureas) can increase the incidence of disease, but such interactions tend to be site-specific and may not be widespread. More research needs to be done to elucidate the mechanisms by which these events occur. However, in the meantime, it is advisable that farmers maintain vigilance as to potential herbicide–disease interactions.

Aside from qualitatively characterizing the hazards posed by herbicides to soil organisms and functions, this review also identified a number of knowledge gaps and issues regarding the framework by which herbicide risks are assessed and the relevance of laboratory-generated knowledge to agronomy and soil science in the field. One of the primary issues is that, unlike most terrestrial or aquatic ecotoxicological studies involving higher plant and animals, traditional dose–response analyses for describing herbicide impacts on soil microbial communities may not be appropriate or sufficient for describing the environmental hazard. This is for a number of reasons.

First, standardized response variables or endpoints are lacking and single response variables are unlikely to capture all potential hazards. In the context of soil health and ecology, such variables can include measures of biological (community) structure or measures of function. Since numerous methodologies exist for both structural and functional characterization of soil biological communities, there is a major lack of consistency between studies, which makes interpretation and generalization of results difficult. Moreover, because the role and ecology of the majority of microbial taxa remain unexplored, we currently lack the means to extrapolate measures of microbial community structure to specific ecosystem and agronomic functions; and vice versa.

Second, even if significant shifts in a microbial community occur in response to a herbicide input, functional redundancy within the community means that such shifts may not translate to a loss of function (that is, the soil exhibits a functional resistance). Compounding this fact is that the pace of physiological and evolutionary adaptation of microorganisms to disturbance is much faster than in higher ecological systems, so that an ecological disturbance caused by repeated herbicide inputs may be overcome or circumvented (that is, the soil exhibits a functional resilience). On the other hand, there is a possibility that nonsignificant community or functional shifts may still decrease the *capacity* for further resistance or resilience against other stressors, as has been observed for higher organisms (eg,

Bandowet al., 2014). To our knowledge, this issue has not been directly assessed with reference to herbicides and requires attention.

Third, what is the relevance of changes to structural and functional response measurements? Certain response measures, particularly those which are general descriptors of microbial diversity or microbial activity, such as respiration, dehydrogenase activity, or hydrolase activity, provide ambiguous evidence for “negative” impacts to soil health. In many cases it cannot be determined whether increases or decreases in these measures are a consequence of stress toward microbial cells; increased turnover of microbial cells due to cell death and lysis; specific degradation of the herbicide input; or potential priming and increased degradation of indigenous organic matter. Depending on the agronomic situation, a reduction in a particular function may be beneficial or detrimental. A prime example is that of nitrification: under high nitrogen loading, inhibition of nitrification is sometimes desirable in order to reduce subsequent leaching or denitrification loss of N. In comparison, inhibition of nitrification under low soil nitrate availability can reduce crop N uptake and productivity.

An additional constraint to our understanding of the impact of herbicides on soil functions contributing to crop/pasture productivity is that it is difficult to translate the results of short-term spatially-confined laboratory experiments into a long-term field perspective. Many of the response indicators used to assess soil health are “snapshot” measures, such as enzyme activities, that are only measured periodically at (usually) arbitrary time points. Since microbial dynamics vary on timescales of hours to days, it is difficult to assess the impact of a short-term stimulation or inhibition over a cropping season or longer. For example, conclusions from short experiments on the impact of a herbicide thoroughly mixed through soil and maintained at constant water content cannot be directly applied to field conditions where the same herbicide is applied to the soil surface, which has a stratified organic matter profile and soil moisture is highly variable both spatially and temporally. If negative impacts only occur in the zone where the herbicide is present in damaging concentrations (which may only be in the top few millimeters of soil for some herbicides) then does this really influence the capacity of the soil as whole to support plant productivity? Alternatively, is the impact at a time critical to growth and nutrient supply? Hazards identified in dose–response studies therefore need to be translated to risks in the field, via modeling that incorporates temporal and spatial aspects, such as herbicide redistribution and dissipation, microbial population evolution and adaptation, and physicochemical changes within the soil profile.

Therefore there is a strong need for a consistent framework for assessing meaningful endpoints to agricultural production systems. Such a framework needs to integrate the extent and duration of disruption to critical processes. We recommend that future studies in this area of research should:

- Report the concentration of herbicide applied to soil as both a field rate (ie, kilogram herbicide per hectare) and a mass concentration (ie, milligram herbicide per kilogram of soil);
- Utilize a range of techniques to give a comprehensive picture given the inherent bias in some techniques for determining changes in soil biological community structure and function;
- Aim to better understand the effect of commercial formulations relative to active ingredients;
- Attempt to link the findings of laboratory incubations to field situations through additional semifield or field studies, or complementary modeling to extrapolate impacts to realistic field scenarios.

It is our intention that this review, although not fully exhaustive, provides a rigorous starting point from which future studies can improve our understanding of the potential impacts of herbicides on soil biology and function.

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Performance of Coffee Seedlings as Affected by Soil Moisture and Nitrogen Application

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Abstract

Nitrogen (N) and soil moisture are the most important factors controlling yield in Colombian coffee crops. Since long-term productivity is contingent on robust early growth, it is imperative to study these factors in seedlings in order to ensure maximum yield potential of mature trees. A greenhouse experiment with four soil water and four N levels was used to determine how these two important variables affect quantifiable parameters representing seedling performance. Shoot biomass, the most obvious indicator of performance, was increased by both higher soil moisture and higher N application, although root biomass increased with N application only in wetter soils and in general the root to shoot ratio diminished with increasing N application. Like shoot biomass, leaf N content and N derived from fertilizer (Ndff) also increased with increasing N application, with relatively more leaf N recovered from fertilizer as soil moisture increased. Evapotranspiration (EVPT) responded to soil moisture, whereas water use efficiency, in terms of shoot mass produced per unit of evapotranspired water, responded dramatically to N level but was not affected by soil moisture. The strong effect of N application on water use efficiency was affirmed by the higher $^{13}\text{C}/^{12}\text{C}$ ratios and stem water potentials of plants grown under higher N levels, indicating greater water stress in these plants. All of these responses were associated with changes in photosynthesis as a result of acclimation to the imposed conditions. Consideration of these results will facilitate new recommendations for maximizing growth of coffee seedlings by increasing water and N use efficiency while reducing the economic and environmental impacts attributed to N fertilizers.



1. INTRODUCTION

Water and N fertilizer are usually supplied in excess to crops to increase yields, but this has led to a simplified intensification of practices to achieve maximum yield potential. Such an approach to management is embodied in the notion that the intensification of agriculture endeavors to maximize production and minimize the cost of food with little regard to impacts on the environment and the services it provides to society and nature (Tilman, 1999). Current strategies for sustainable resource utilization in agriculture necessarily include both efficient use of resources as well as a reduction in the environmental impact of management practices, particularly those associated with fertilization. Coffee (*Coffea* sp.), one of the major crops cultivated in tropical areas, is managed with nitrogen (N) application rates that vary between 200–300 kg N ha/year (Bornemisza, 1982; Sadeghian, 2008). These application rates can result in loss by volatilization of at least 30% of a broadcast application of urea, the most common N fertilizer and application practice used in coffee production (Leal et al., 2010). In addition, from

30 to 55% of the applied N can be leached as NO_3^- beyond the root zone (Cannavo et al., 2013). Although coffee plants may reabsorb up to 43% of the volatilized gaseous ammonia (NH_3) (Fenilli et al., 2007), recovery of urea N in young plants averaged only 5%, regardless of whether it was broadcast or incorporated (Salamanca-Jimenez, 2015). Such data indicate that most of a fertilizer N application is lost from the root zone, and therefore any positive effect on crop yield is only slight compared to negative environmental and economic impacts that have yet to be accurately determined. In Colombia, an important coffee-producing area, N fertilization as broadcast urea is done during the anticipation of rain events to maximize efficiency and nutrient uptake by the coffee crop (Sadeghian et al., 2014). However, as Rosenthal (2011) stated in a newspaper article, changes in coffee yield are occurring all over the world due mainly to climatic variability likely resulting from global warming; in Colombia, for example, more intense heavy rains coupled with longer drought periods are harming crops and farmers across the country. As rain distribution patterns change through time, according to DaMatta and Ramalho (2006), areas where coffee is cultivated will be greatly affected either by water deficits or excesses, creating less-favorable conditions for production quality and lower profitability that may force farmers to expand onto new land, likely at the expense of native forest (Schroth et al., 2009); farmers may also be subject to confounding ideas about the timing of key agricultural practices such as fertilization (Lobell and Gourdj, 2012).

The possibility of increased changes in growing conditions is highly relevant because successful cultivation of coffee in Colombia is associated with a unique environmental context: the most productive coffee region is located between 1000 and 2000 m above sea level, where the mean annual temperature is between 15 and 23°C and annual precipitation between 1000 and 4000 mm (Jaramillo, 2005). Photosynthesis is the physiological process in coffee which is most dependent on optimal conditions (Lopez, 2004), and will likely be impacted as a result of future climate change.

Photosynthesis or net assimilation in coffee is affected by multiple factors such as radiation, air temperature, relative humidity (Kumar and Tieszen, 1976), shade level, fruit load, fertilization and water deficit (DaMatta, 2004). Optimal photosynthesis and growth, which rely on adequate N supply, reduce photoinduced damage to plants (DaMatta, 2004), while it has been stated that drought (DaMatta and Ramalho, 2006) and temperature (Gay et al., 2006) are among the major limitations for coffee production; both of these climatic factors affect water availability. Tesha and Kumar (1978) discuss how additional N increases resistance to drought, but to date

no studies have examined the connection between water availability and N fertilization in Colombia.

Given that high N doses applied during the vegetative stage often lead to low N use efficiency (NUE), it is imperative to understand the nutritional requirements of seedlings to ensure maximum yield potential of reproductive coffee trees. According to [Nielsen \(2006\)](#), NUE is influenced by both crop photosynthetic capacity or growth potential, and by the combination of frequency and severity of N loss events within the N cycle; the desirable goal is always to reach higher NUE values, which means more of an application of N has been captured by the plant and less has been lost to the environment.

Current fertilization recommendations for coffee crops emphasize the reproductive stage, which is the most studied and best understood phase. In contrast, the vegetative stage has been less studied as it is not associated with a direct economic impact (income). Available criteria and guidelines for N application at this stage of growth are therefore poorly defined and generally scarce worldwide. However, early growth stages are critical, since according to [Salazar \(1996\)](#), if seedlings are small when they are transplanted into the field, productivity will suffer for the remainder of the crop cycle.

In light of the importance of early seedling growth and the lack of relevant and applicable knowledge, the present study sought to investigate how N application and water availability affect quantifiable parameters representing coffee seedling performance. More efficient use of N is the most pressing goal, and therefore it is necessary to better understand how N utilization and water availability together affect coffee productivity. This understanding can be used to improve recommendations for maximizing growth of coffee seedlings, by using critical resources more effectively while at the same time reducing the impacts attributed to N fertilizers, and will contribute to improved sustainability and productivity of coffee crops.



2. MATERIALS AND METHODS

We conducted a greenhouse experiment at the University of California, Davis, between September 2011 and June 2012 using 3-month-old coffee (*Coffea arabica* L) grown from seed of cv line CX 2720. Seedlings were maintained in 2.3 L plastic pots containing soil representative of typical coffee production areas in Colombia (Andisol) mixed with silica fine sand #60 in a 4:1 soil: sand ratio. Some chemical properties for the soil are presented in [Table 1](#).

Table 1 Chemical properties of the soil used.

pH		OM	Olsen P	NH ₄ –N	NO ₃ –N	K	Ca	Mg	CEC
Water	KCl	g/kg	mg/kg			cmol+/kg			
6.7	5.5	160	4	2.1	1.2	0.62	9.6	1.45	13.7

Sand was used to enhance the physical properties of the substrate and a typical analysis from [Axner \(2014\)](#) shows that this contained 75.0–84.0% of SiO₂, 8.0–12.0% of Al₂O₃, 0.2–0.6% of Fe₂O₃, 1.0–4.0% of Na₂O, 1.0–3.0% of CaO, 0–0.1% of MgO, 3.0–5.0% of K₂O, and 0–0.3% of Ti₂O.

2.1 Treatments

From the results of a previous experiment under similar conditions ([Salamanca-Jimenez, 2015](#)), we established that the best moisture levels to be evaluated in terms of soil matric potential were in the range of –10, –50, –100, and –500 kPa, which correspond respectively to volumetric water contents of 28, 22, 17, and 13%, or 117, 92, 71, and 54% of field capacity. These data were obtained from a soil water retention curve determined with the pressure plate method. From the same experiment, we established that N doses above 0.4 g/plant caused a detrimental effect on plant growth and reduced NUE. For this reason, we determined that the best N doses to consider were 0, 0.1, 0.2, and 0.4 g/plant. In the present experiment, N doses were split into four applications, applied every 2 months as ¹⁵N-labeled urea with an enrichment of 1.0 atom percent ¹⁵N.

We also included two levels of potassium in order to determine if this nutrient could have a significant effect, especially in regards to tolerance to water stress. However, likely due to the fact that soil K level was increased from the sand component, the applied dose (0.2 g/plant) was not enough to show any significant effect. At the end of the experiment this level of variation was excluded from the analysis and the data were combined to include only two sources of variation: water and N. Although the initial design when K was included was a split plot with 5 replicates, this became a randomized block design with a 4 × 4 factorial and 10 replicates when K was excluded.

2.2 Measurements

Each replicate (plant) was watered every 3 days using a syringe connected to a drip system located next to the tap root extending along the upper 15 cm of the pot. The design applied water uniformly across the imposed soil matric

potentials. Water loss was replaced according to the specific target weight defined by each matric potential. The water applied never drained from any pot and the wetting zone of the driest soil treatment was never distributed over the whole mass of soil. Instead, the confined wetted area was enough to show effects on plant growth and all the measured variables. The total loss of water through evapotranspiration (EVPT) during the experimental period was calculated as the total amount of water applied to each individual replicate, accounting for the remaining soil moisture at the end of the experiment.

During the last 3 months of the trial we measured physiological variables between 9 am and 2 pm on a monthly basis. This was done on the first pair of fully expanded leaves from the top either 1 or 2 days after watering, using the auto log mode of a LI-6400 portable gas exchange system (LI-COR). The details of the calculations of the measured parameters reported by the LI-COR equipment are given by [von Caemmerer and Farquhar \(1981\)](#). The measured LI-COR chamber set-up parameters were based on a flow rate of 500 $\mu\text{mol/s}$, a CO_2 concentration of 400 ppm, a PAR of 700 $\mu\text{mol photons/m}^2\text{s}$, a block temperature of 22°C and a relative humidity between 50 and 80%. All LI-COR data were averaged as repeated measurements on each plant, and final photosynthesis (A), stomatal conductance (g), internal CO_2 concentration (Ci), transpiration (T), and leaf-to-air vapor pressure deficit (VPD) were analyzed according to a factorial design.

We also measured stem-water potential (stem Ψ) 1 day before harvesting all replicates using a pump-up pressure chamber following the methodology described by [pmsinstrument.com \(2015\)](#). One fully expanded leaf from the top was enclosed within an aluminum bag and after 15 min, this leaf was cut and put inside the chamber to measure the water potential.

At the end of the experiment, we determined the level of chlorophyll *a* and *b* as well as carotenoids in the leaves by extraction with dimethylsulfoxide (DMSO) followed by spectrophotometric measurement at wavelengths of 665, 645, and 480 nm respectively, as described by [Lichtenthaler \(1987\)](#). Chlorophyll levels were also determined using a Minolta SPAD meter, avoiding the major leaf veins, to compare with the lab analysis and establish the relationship among these measurements. The use of this meter has been promoted in different crops as a diagnostic aid to establish N deficiencies in the field and make quick decisions about N fertilization requirements. Details about the specifications and calibration of the meter are reported by [Markwell et al. \(1995\)](#).

After 9 months, when plants had reached a size similar to that of plants transplanted in the field, all replicates were harvested and separated into their

components (leaf, stem, root). Foliar area was measured immediately using a scanner and quantified with ImageJ Software using a known area as a reference. Each component was dried and weighed, but only leaves were milled. Subsamples were sent to the Stable Isotope Facility at UC Davis where the N content as well as the amounts of ^{15}N and ^{13}C (the latter as an integrative measure of water stress) were measured by stable isotope ratio mass spectrometry. The percent of plant N derived from fertilizer (Ndff) and the percent of fertilizer N recovered in plants (NUE) were calculated as follows:

$$\% \text{Ndff} = (\text{atom\% excess } ^{15}\text{N in leaf sample} / \text{atom\% excess } ^{15}\text{N in fertilizer}) \times 100$$

$$\text{NUE} = [(\text{mass N in leaves}) \times (\% \text{Ndff} / 100)] / (\text{mass fertilizer N applied})$$

Water use efficiency (WUE) was calculated as g of shoot biomass relative to total water applied (EVPT).

2.3 Statistical Analysis

As mentioned previously, this experiment was initially designed to test the effect of 32 treatments consisting of two levels of K, four levels of soil matric potential and 4 N rates ($2 \times 4 \times 4$ factorial) on dry biomass (leaves, stems, and roots), total N uptake, NUE and WUE and physiological traits of coffee seedlings. However, because no effect of the K factor was observed we proceeded to analyze the data as a randomized complete block experiment with a 4×4 factorial design consisting of 10 blocks and 16 treatments per block, where the experimental unit for testing treatment effects was one coffee plant.

Seedlings were blocked according to their size at the age of 3 months, before the beginning of the experiment. The effects of soil matric potential and N rate on each parameter were assessed using a general linear model. Block, soil matric potential, N rate, and soil matric potential by N rate interaction were included in the model as fixed effects. Model fit was assessed for each parameter by using the Kolmogorov-Smirnov and Levene's test for normality of residuals and homogeneity of variances, respectively; since both of these tests were not met, a subsequent data transformation using the power option was undertaken as it was the only transformation that met the criteria required for both tests. Results were declared to be statistically significant at the 5% level and all analyses were performed using SAS for Windows Version 9.2 (SAS, 2009).

Means and standard errors of all parameters were calculated for each treatment, and the effect of water and N levels as well as the water and N interaction were estimated across all blocks and treatments using a two-way ANOVA. For those cases in which the interaction was significant, we used response curves to help explain the interaction as described by [Montgomery \(2012\)](#). When appropriate, pairwise comparisons or estimates of simple effects were used to further analyze interaction effects. In the case of no interaction, main effects were analyzed using Tukey's post hoc test.



3. RESULTS

Main effects of water or N were observed on N derived from fertilizer (Ndff), NUE, content of chlorophyll *b* and carotenoids, photosynthesis, stomatal conductance, transpiration, and leaf-to-air VPD, whereas an effect of the interaction between water and N was observed on plant biomass, root to shoot ratio, foliar area, leaf N content, chlorophyll *a* content, ^{13}C composition, WUE, total EVPT, and stem water potential.

3.1 Plant Performance as Indicated by Plant Growth, Root to Shoot (R:S) Ratio and Foliar Area

The average root and shoot biomass as well as the R:S ratio and foliar area are presented in [Fig. 1](#). There was a significant effect of the interaction between soil moisture and N on root and shoot biomass ($p < 0.0001$, $p < 0.0001$). The shoot biomass, the most obvious indicator of performance, was increased by both higher soil moisture and higher N rates. The highest shoot biomass was registered for the wettest soil (-10 kPa) when plants were fertilized with 0.4 g of N, whereas the lowest biomass corresponded to the driest soil (-500 kPa) with no N. The highest root biomass was registered in the wettest soil and 0.4 g N/plant whereas plants growing in the driest soil with 0.2 g N per plant exhibited the lowest root mass.

On the other hand, an effect of the interaction of water with N was registered on root to shoot ratio ($p = 0.0024$), which decreased with increasing N; R:S tended to increase as soil moisture decreased but this trend was not statistically significant. Foliar area exhibited an effect of the interaction of water with N ($p < 0.0001$) similar to the biomass, both positively affected by increasing N but negatively affected by decreasing soil moisture.

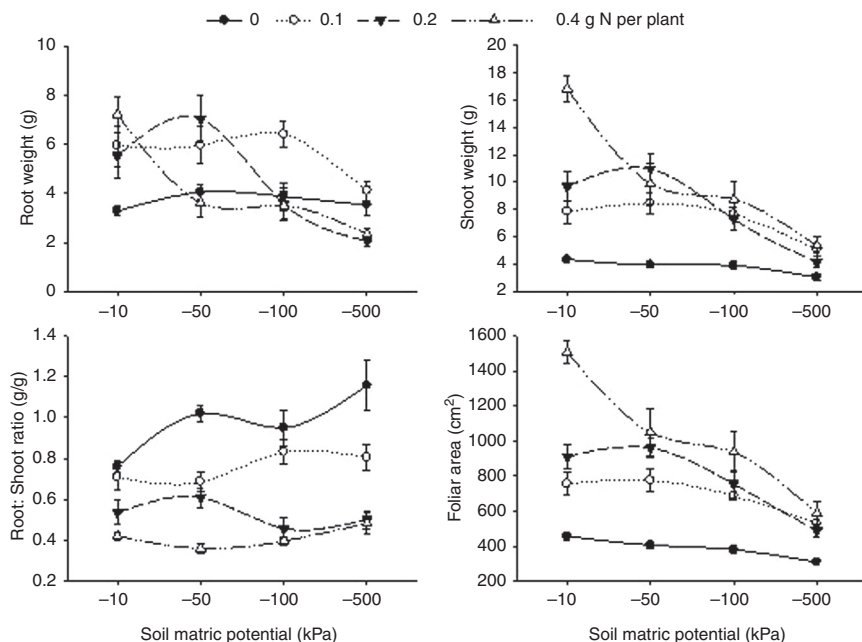


Figure 1 Biomass allocation in coffee seedlings grown under different soil moisture and N levels.

3.2 Performance as Indicated by Leaf N Content, Ndff and NUE, and Chlorophyll Content

The variables related directly to N uptake are presented in Fig. 2 whereas chlorophyll data are presented in Fig. 3. An effect of the interaction of water with N was registered on leaf N content ($p < 0.0001$), whereas only main effects of N were registered on Ndff ($p < 0.0001$) and of water on fertilizer use efficiency ($p = 0.0019$). All the controls without N exhibited similar leaf N content, but N application increased N uptake and this effect was more pronounced in drier soil with higher N doses. In addition, the Ndff in the leaves increased with N dose but was not affected by soil moisture levels. In contrast, N recovered from urea (NUE) increased with increasing N dose in the wettest soil, remained unchanged at -50 and -100 kPa, and decreased with increasing N dose in the driest soil but without a significant interaction ($p = 0.1730$). These results are opposite to those of a previous experiment with higher N doses and older plants (Salamanca-Jimenez, 2015), where it was shown that NUE decreased with increasing N availability regardless of soil water content.

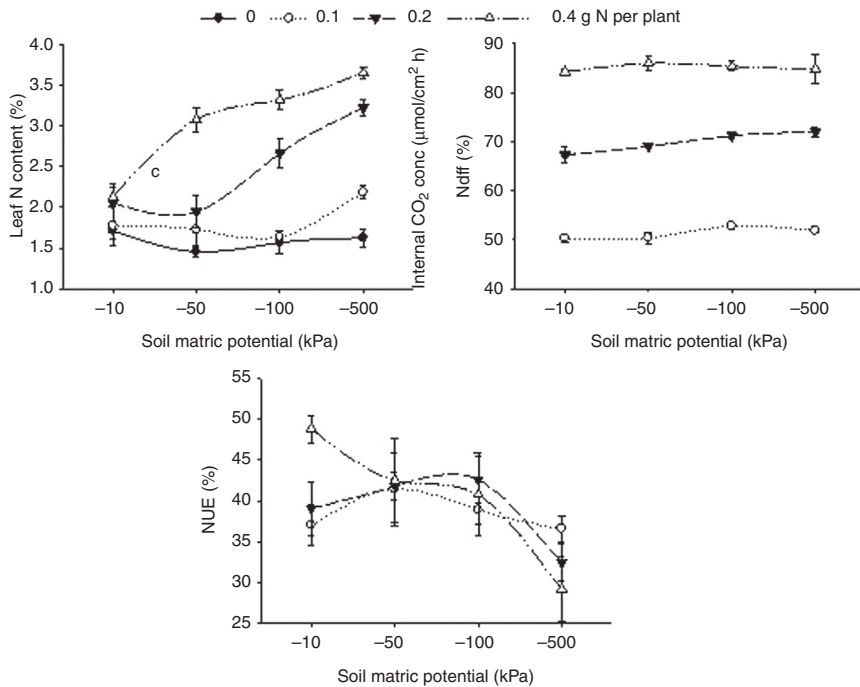


Figure 2 Leaf N uptake in coffee seedlings grown under different soil moisture and N levels. Ndff, N derived from fertilizer; NUE, fertilizer N use efficiency.

The content of chlorophyll *a* in leaves was also significantly affected by the interaction of water with N ($p = 0.0327$), although chlorophyll *b* and carotenoid content exhibited only main effects in response to levels of water and N ($p < 0.0001$, $p < 0.0001$) (Fig. 3). The three pigment variables increased as N increased at each water level and were higher overall when water soil decreased. Likewise, the SPAD readings with the chlorophyll meter were affected by the interaction of water with N ($p = 0.0044$) and were also related to both the N and chlorophyll contents in the leaf. Control plants without N exhibited similar SPAD readings across water levels and N addition caused these readings to increase proportionally, being highest for plants in the driest soil receiving 0.4 g N per plant.

In contrast to concentration on an area basis (Fig. 3), when the total mass of leaf pigments was calculated (Fig. 4), we observed a highly significant effect of the interaction of water with N ($p < 0.0001$) on chlorophyll *b* and carotenoids and only a slight effect on chlorophyll *a* content ($p = 0.0463$). The control plants without N accumulated a similar amount of pigments at

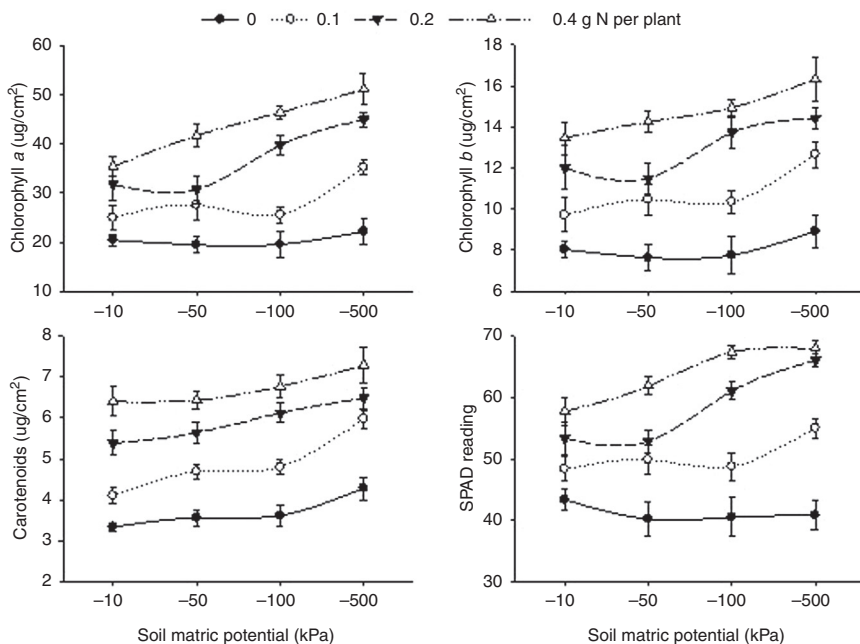


Figure 3 Leaf chlorophyll and carotenoid content in coffee seedlings grown under different soil moisture and N levels.

all water levels but this increased proportionally with N addition. Coffee plants fertilized with 0.4 g of N had the highest total pigment content at each water level. The driest soil (−500 kPa) exhibited the lowest amount of pigments among water levels compared to other N doses and no differences were registered for the intermediate N doses among the other water levels. In addition, a comparison between the SPAD readings and the amount of each pigment per unit area shows a linear relationship with high correlation coefficients.

3.3 Performance of Seedlings as Reflected in WUE, ¹³C Composition, Total EVPT and Stem Ψ

Data for WUE, ¹³C composition, total EVPT and stem water potential (Ψ) are presented in Fig. 5. There was a significant effect of the interaction of water with N on both WUE ($p = 0.0134$) and ¹³C content in the leaves ($p = 0.0208$). WUE and ¹³C content increased dramatically with increasing N doses, reaching the highest values at a soil moisture equivalent to

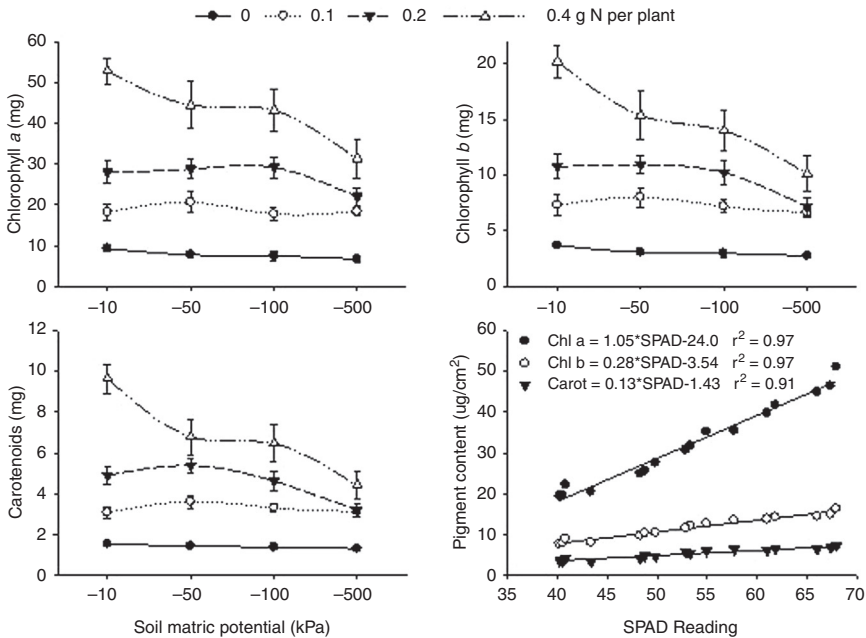


Figure 4 Total amount of leaf pigments in coffee seedlings grown under different soil moisture and N levels, and relationship between chlorophyll meter (SPAD) reading and total pigments.

−500 kPa and the maximum N rate (0.4 g/plant). The value of $\delta^{13}\text{C}$ was taken to indicate relative water stress and largely mirrored WUE, a measure of the amount of ET required to produce the shoot biomass. The same figure shows that there was a significant effect of the interaction of water with N on EVPT ($p = 0.0008$) and stem Ψ ($p = 0.0086$): the effect of N dose on ET was diminished in drier soils. As might be expected, stem Ψ also responded to our treatments, decreasing with soil moisture and showing lower values as N addition increased. This response, reflecting the effects of both soil moisture and N fertilization, resulted in a strong correlation between stem Ψ and ^{13}C content (Fig. 6).

3.4 Physiological Traits as Indicators of Plant Performance

Most of the responses presented previously were associated with changes in photosynthesis as a result of acclimation to the imposed conditions (Fig. 7). A significant effect of the interaction of water and N was registered only on the intercellular CO_2 concentration ($p = 0.0122$), while main effects of soil

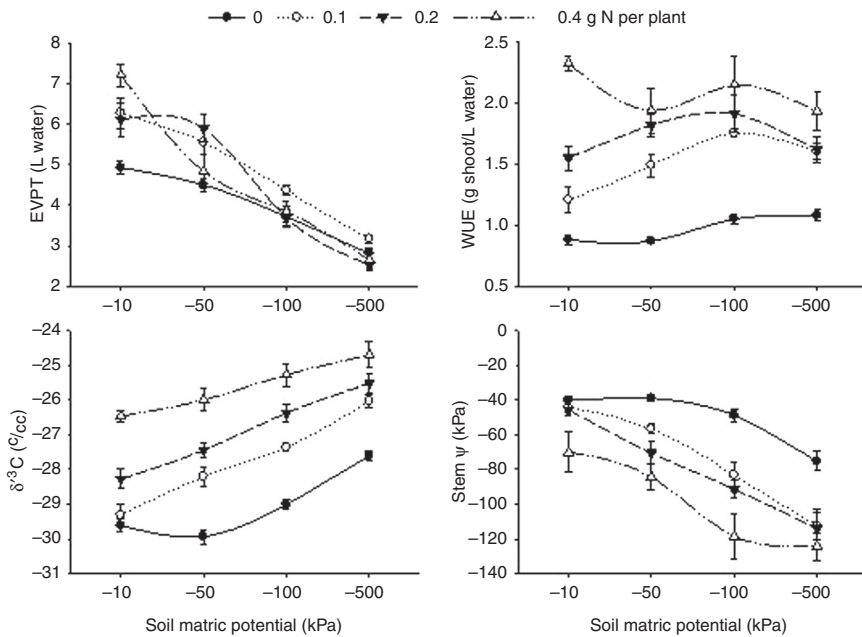


Figure 5 WUE (water use efficiency), EVPT (evapotranspiration), carbon isotope composition ($\delta^{13}C$), and stem water potential (Stem Ψ) in coffee seedlings grown under different soil moisture and N levels.

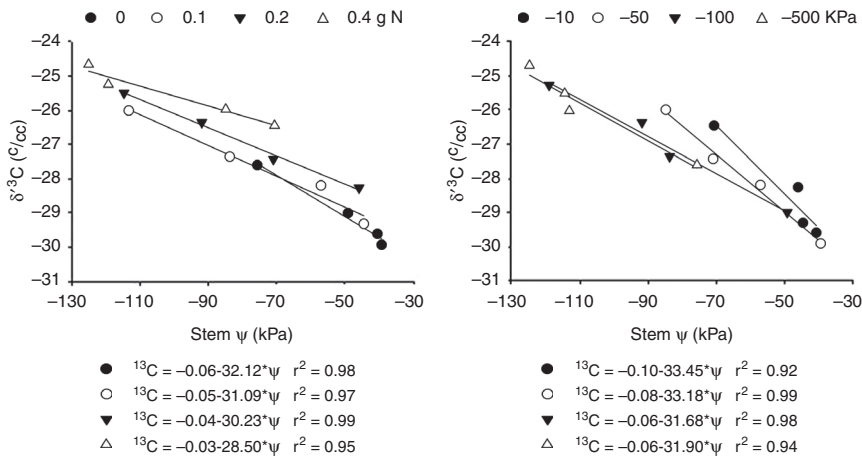


Figure 6 Linear regression between stem water potential (Ψ) and $\delta^{13}C$ content in coffee seedlings by N and soil moisture levels.

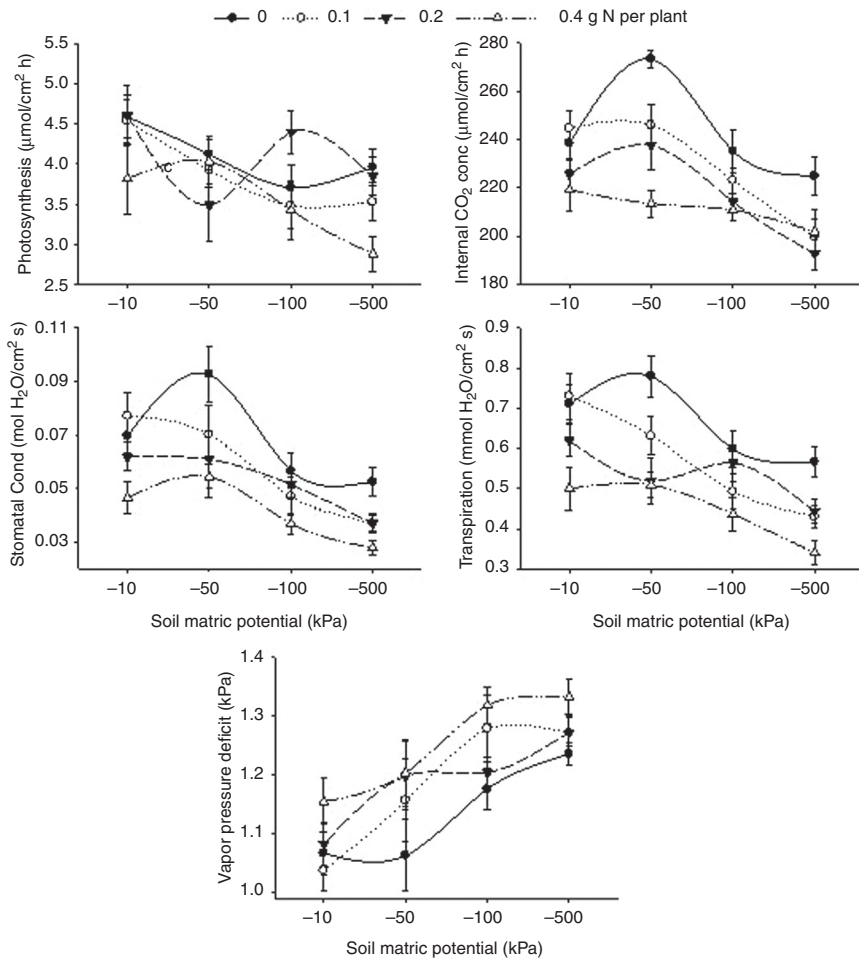


Figure 7 Physiological traits in coffee seedlings grown under different soil moisture and N levels.

moisture and N were registered on rate of photosynthesis ($p = 0.0014$, $p = 0.0344$), stomatal conductance ($p < 0.0001$, $p < 0.0001$), transpiration ($p < 0.0001$, $p < 0.0001$), and leaf-to-air VPD ($p < 0.0001$, $p = 0.0005$). In general, photosynthesis, internal CO_2 concentration, stomatal conductance and transpiration were higher in the unfertilized plants and decreased with increasing N dose. The lowest values for these parameters corresponded with water stress induced by drier soil conditions, which at the same time increased the VPD in the leaves concomitant with the N fertilization.



4. DISCUSSION

4.1 N Fertilization and Growth of Young Coffee Plants

Almost all research on coffee N fertilization has focused on the economically tangible reproductive stage, but few studies have considered the early vegetative stage, even though this stage is critical to a plant's future performance. [Sadeghian \(2008\)](#) concludes that insufficient N fertilization during the vegetative phase decreases future bean yields up to 50%. The most basic results of our study of seedlings, from an agronomic point of view, confirm the importance of N from the beginning of coffee production. Shoot biomass and foliar area responded positively to N at all levels of soil moisture, but the root response did not follow a marked trend line as had been found in a previous experiment in which N application decreased root biomass ([Salamanca-Jimenez, 2015](#)). Our results highlight the interaction between N fertilization and soil moisture, in particular, the importance of soil moisture in increasing N uptake. Under higher moisture, NH_4^+ and NO_3^- move with greater ease to roots and are taken up mostly by transpiration-driven mass flow ([Marschner and Rengel, 2012](#)).

We observed that wetter soils allowed the roots of fertilized plants to explore a greater volume of soil. This may partly explain why the growth of plants fertilized with 0.4 g N/plant was enhanced at higher soil water contents. Conversely, the root to shoot ratio (R:S) was reduced by increasing N dose and increased by withholding soil moisture. Similar results were reported by [Bravo and Fernandez \(1964\)](#) who observed that coffee plant growth following N application exhibited a linear increase as available water increased; they also reported that R:S was reduced by N fertilizers but was not, however, affected by soil moisture.

In general, the optimal combination of water and N for coffee growth was the wettest soil (−10 kPa) and 0.4 g N per plant. [Sadeghian and Gonzalez \(2014\)](#), working in one Colombian soil with 13% organic matter, estimated that a dose of 0.54 g N per plant would produce the highest coffee seedling biomass, which is similar to the rate of 0.48–0.6 g recommended by [Arizaleta et al. \(2002\)](#). In contrast to our results which showed an improvement in seedling performance with increasing N dose, [Giraldo and Rubiano \(1974\)](#) and [Salazar \(1977\)](#) registered a negative effect of N on coffee growth during the seedling stage, although it is possible that the doses used in these studies exceeded beneficial levels – from a previous experiment, we also

observed that excessive application of N (0.8 and 1.2 g N/plant) became harmful for coffee growth and reduced NUE (Salamanca-Jimenez, 2015).

Although Arizaleta and Pire (2008) state that plant response to N is associated with soil fertility, we believe that it is more related to plant N requirements. For this reason, if the goal of fertilization management is to maximize coffee growth by increasing NUE, N doses must not exceed 0.6 g N per plant and fertilization during the vegetative phase should be adjusted proportionally to this optimum value.

4.2 Leaf N Content, Ndff and NUE, and Chlorophyll Content

We observed a positive response of increasing N in leaf N content, Ndff, leaf pigments and SPAD readings. Similarly, Bravo and Fernandez (1964) showed that foliar N and chlorophyll content in coffee increased as soil moisture decreased under greenhouse conditions. The same effect was noted by Quaye et al. (2009) in corn, who found that the highest N rate (80 kg/ha) caused a significant increase in biomass yield and N uptake with moisture levels close to field capacity.

Unlike leaf N, Ndff, or leaf pigments, the percent of N recovered from fertilizer (NUE) did not exhibit a clear response to treatment conditions. Only plants grown in the wettest soil and with the highest N dose showed an increase in NUE with increasing N, but no response was observed for the other soil moisture levels. This behavior is opposite to that found in a similar experiment (Salamanca-Jimenez, 2015) where NUE was decreased by increasing N doses, although in this case it was likely due to excessive N.

The absolute values of Ndff and NUE in the present study were very similar to those presented by Salamanca-Jimenez (2015), confirming that the magnitude of the N uptake response within certain limits is an intrinsic characteristic of coffee seedlings. Nevertheless, NUE values obtained in pot studies may be higher than those representative of the field, as roots are confined in pots and N is typically applied directly to the root system with no leaching permitted. Much lower NUE values in coffee have been reported under field conditions by other studies using ^{15}N : Cannavo et al. (2013) registered an N recovery of 13.5% in the whole plant and Salamanca-Jimenez (2015) reported an average of 3% in the leaves. In both studies, these low NUE values were associated with considerable loss of N either by volatilization or by leaching in places with high precipitation. Similarly, Suarez (1996) studying the effect of four N levels (6, 12, 18, and 24 g N/plant/year) in a high density (10,000 plants/ha) reproducing coffee plantation established on an Andisol, found that only 8% of fertilizer N was taken up, exhibiting the following

distribution: 59.7, 21.7, 9.9, and 8.8% in leaves, branches, stem, and roots, respectively.

Riaño et al. (2004), evaluating N uptake and dry matter accumulation in coffee plants during the first 15 months after transplanting in three Colombian fields, found that N uptake reached 8.6 and 19.4 g per plant. Despite being similar to the 27 g N per plant in 24 months reported by Suarez (1996), this reflects a very low NUE, considering that one coffee plant receives between 47 and 58 g of N fertilizer, not including native soil N or previous applications.

Our study shows that the amount of N addition was more important than soil water content for chlorophyll and carotenoid concentrations and total content in coffee leaves. According to Lichtenthaler (1987) chlorophyll *a* and *b* occur in a ratio *a/b* of approximately 3 but growth conditions and environmental factors can modify this ratio. For example, plants growing under high levels of sunlight (high-light chloroplasts) exhibit *a/b* ratios of 3.2–4, whereas shaded plants (low-light chloroplasts) possess lower values (2.5–2.9). The *a/b* values in our plants averaged between 2.4 and 3.1, confirming that our plants reflected a physiology typical of low light conditions.

It has also been stated that a ratio of chlorophyll *a+b*/carotenoids lower than 3.5 can be used as an indicator of senescence, stress, and damage to the plant and the photosynthetic apparatus, since chlorophyll pigments break down faster than carotenoids (Lichtenthaler and Buschmann, 2001). In our study this ratio averaged 8.5, corroborating favorable conditions for high photosynthetic activity in the top fully expanded leaves of coffee plants, and was affected more by increasing the N level than by increasing soil moisture. Lichtenthaler (1987) also states that the individual levels of pigment proteins also change depending on the developmental stage and the environmental conditions, which again explains the higher values observed for the ratios chlorophyll *a*/carotenoids and chlorophyll *a+b*/carotenoids in low-light plants. Carotenoids play an important role in the use of solar energy in photosynthesis, due to their ability to safely dissipate excess energy (Demmig-Adams and Adams, 1996).

4.3 Changes in WUE, EVPT and Stem Water Potential (Stem Ψ)

Our results confirm the positive effect of N on WUE and the negative effect of soil water level on EVPT and stem Ψ . When plants grow under water stress they use available water more efficiently, but as soil moisture decreases the water potential differences between the soil and the environment increase and limit water movement both to and through the plant. In

addition, water-stressed plants close their stomata, which results in an increase in ^{13}C content such as that seen in our study.

Quaye et al. (2009), evaluating N rates combined with soil moisture regimes in potted plants, also found that the response of corn to N rate was influenced by soil water availability, as EVPT increased with high N rates under soil moisture levels close to field capacity.

As a component of EVPT, transpiration is important for nutrient transport in the plants; Matimati et al. (2014) state that transpiration increases mass flow of nutrients to roots, especially in low-nutrient soils or where the root system is not well developed. In addition, N availability regulates transpiration-driven mass flow of nutrients from substrate zones inaccessible to roots. Under adequate water availability, therefore, mass flow may partially substitute for root density in providing access to nutrients without incurring the costs of root extension, although the efficacy of mass flow also depends on soil nutrient retention and hydraulic properties. Our results confirm these phenomena in that root growth and EVPT exhibited similar responses to water and N levels.

Stem water potential (stem Ψ) has been established as an accurate and reliable measure of water stress in prune trees (McCutchan and Shackel, 1992), and has been used to develop diagnostic thresholds in other deciduous trees such as almonds and walnuts (Shackel et al., 1997, 2000). According to Williams and Araujo (2002) stem Ψ in grapevines at midday is correlated with photosynthesis and stomatal conductance as well as soil moisture, leaf gas exchange and water potential in other plant organs, and is a viable method for assessing water status. Their results agree with those of Chone et al. (2001) who found that stem Ψ was a better indicator of both moderate and severe water deficits; these authors proposed use of this parameter for management of both non-irrigated and irrigated vineyards. In coffee no data exist for stem Ψ except one study done using a PSY1 stem psychrometer by Doney and Arias (no date) (s.f.) during 45 days in a single productive plant. They found that by decreasing soil moisture, stem Ψ also decreased. Our study affirms the value of this variable as an indicator of water stress in coffee plants and may prove useful where drought conditions are becoming prevalent, in order to predict possible effects on the photosynthetic apparatus or establish possible water requirements. It may even be used to predict future blooming periods since it is well known that initiation of blooming is a process which relies on short soil water deficits. As Doney and Arias (s.f.) state, identifying excessive stress is important to either manage or prevent blooming events.

4.4 Response of Physiological Parameters to Soil N and Moisture

Nitrogen and water status both played a crucial role in coffee seedling physiology. In general, most of the measured traits were affected negatively as N increased, with a less pronounced effect caused by soil moisture levels.

According to [Kumar and Tieszen \(1976\)](#), maximum net assimilation (A) in coffee is reached at around 600 $\mu\text{mol photons/m}^2\text{s}$, an air temperature between 20 and 25°C, and relative humidity close to 80%. Our experimental environment met these criteria, implying that no limiting conditions occurred during the experiment.

[Lopez \(2004\)](#) found that the rate of photosynthesis in coffee plants is affected by altitude; higher net assimilation of CO_2 was registered at higher elevations associated with lower irradiation, air temperature, and VPD as well as higher relative humidity. In addition, physiological responses in coffee are affected by shade level, fertilization, fruit load, and water deficits, and N is associated with specific mechanisms of acclimation to reduce photoinduced damage when young plants are transplanted from the nursery to open fields under full sun ([DaMatta, 2004](#)). [Ramalho et al. \(1997\)](#) observed that young coffee plants (1.5–2 years old) with N fertilization and exposed to high irradiation exhibited changes in photosynthesis and pigment composition: protein content increased after 1.5 h at a photon flux density of 1500 $\mu\text{mol/m}^2\text{s}$ and N availability was a key factor during acclimation to high light intensity. Various mechanisms operate during such acclimation; [Ramalho et al. \(2000\)](#) cite in particular an increase in the activity of the antioxidant system and photoprotective pigments (eg, lutein and neoxanthin), as well as changes in the fatty acid composition of chloroplast membranes.

Since the unfertilized plants in our study exhibited higher values of stomatal conductance and transpiration compared to plants with increasing N additions, it is clear that N plays an important role in the response of young coffee plants to water stress, that is, tolerance or acclimation to dry soil conditions.

Other findings in *Coffea arabica* reported by [DaMatta \(2004\)](#) indicate that stomatal conductance decreases as humidity decreases and that during the warmer periods of the day, if soil water is not limiting, an artificial elevation of relative humidity seems to stimulate the stomata to open. Thus, the stomata may respond to changing evaporative demand irrespective of leaf water status. This efficient stomatal closure would restrain photosynthesis while allowing a favorable water status to be maintained for longer periods, thereby ultimately improving survival during prolonged droughts ([DaMatta, 2004](#)).

It has also been found that the site of fertilizer N application also affects the physiological plant response. Bean plants fertilized with N close to the root zone exhibited higher photosynthesis, stomatal conductance, transpiration, and intercellular CO₂, but lower WUE compared to plants fertilized at 45 cm (Matimati et al., 2014); this observation was associated with the regulation by N of transpiration that controls acquisition of nutrients via mass flow. On the basis of these considerations, since coffee plants in our study were fertilized close to the taproot, they likely exhibited maximum rates of transpiration, stomatal conductance, and intercellular CO₂ for each imposed condition.

A study in ryegrass by Luxmoore and Millington (1971), evaluating the effect of N, soil water content, and light intensity, showed that water stress reduced the transpiration rate and the leaf area. Less available water for leaf growth was associated with smaller changes in vapor pressure at the leaf mesophyll surface, since a decrease in plant water potential of 5–10 atmospheres changed the vapor pressure gradient from the mesophyll to the air by less than 1%, independent of changes in stomatal resistance. However, our results show changes in leaf-to-air VPD higher than 10% when plants were fertilized with higher N doses and increases up to 30% under drier soil conditions.

According to Ray et al. (2002), increased VPD leads to reduced WUE, because plants lose more water per unit of carbon gained, and Lobell and Gourdji (2012) further describe that plants respond to reduced soil moisture and very high VPD by closing their stomata, but at the cost of reduced photosynthesis rates and an increase in canopy temperature, which may also increase heat-related impacts. Our results show a similar trend between VPD and WUE, but EVTP, transpiration, stomatal conductance, and Ci values indicate that under high N and low soil water, coffee plants kept their stomata closed for longer periods. Visible heat impacts were not observed under the present experimental conditions, likely because frequent watering events prevented soil moisture from becoming limited enough to cause harm.

In light of our results, a soil moisture potential of –50 kPa can be considered a critical level, since although WUE increases as the soil dries beyond this level, negative effects begin to become apparent in growth, N uptake, and photosynthetic parameters. In cereal crops it is known that water stress, especially during reproductive periods, can be particularly harmful (Hatfield et al., 2011); however, no such differentiations have been made among growth stages in coffee plants.



5. CONCLUSIONS AND IMPLICATIONS

The response of coffee seedlings to different soil water and N levels was primarily due to the relative water stress perceived by the plants. Most importantly, since N use efficiency is only minimally affected by soil moisture, N applications should preferentially coincide with drier soil conditions to reduce environmental impacts caused by leaching. As part of an efficient fertilizer management program, estimates of N requirements in the field or nursery can be quickly obtained with a chlorophyll meter, as such measurements were well correlated to laboratory data in the present study.

Parameters related to plant water use and status showed several strong relationships with each other as well as with N status and photosynthetic performance; some of these measurements may also prove useful in evaluating the fitness of young plants under changing conditions or management strategies. Furthermore, both physiological and morphological parameters may be used as traits for selecting coffee genotypes with superior performance under conditions of water deficit (Vinod, 2012).

The present study is the first to integrate stable isotope data and physiological parameters to examine a critical stage and critical variables in coffee seedling production. The significance of the results, conditions, and parameters considered here can be more thoroughly evaluated as more data are obtained under field conditions and over longer time scales extending into the reproductive phase. Practical application of this knowledge will permit faster development of cultural practices to improve N use and to observe how N uptake and allocation vary as soil and climatic conditions change. As Zapata and Hera (1997) state, information concerning the times and methods of application of different fertilizers is always required, and experiments using isotopes provide a direct and quick means of obtaining conclusive data. For instance, it has been shown that fertilizer rates can be effectively reduced if loss through volatilization is controlled by introducing small changes in management such as gentle incorporation into the soil or mulching with plant residue (Leal, 2006). Finally, any farming practice that proposes to increase N use efficiency by reducing N losses during the vegetative growth stage will reduce the environmental impact attributed to ammonia volatilization and nitrate leaching, and will contribute to making coffee production more economically profitable and sustainable in Colombia and countries with similar issues such as Brazil, Vietnam, and others in Central America and Africa.



6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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